FORMULATION AND EVALUATION OF GELATIN MICROSPHERES LOADED WITH LISINOPRIL DIHYDRATE

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CERTIFICATE

This is to certify that this dissertation entitled

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Constitutes the original work carried out by

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For the partial fulfillment of the requirements for the award of Degree of Master of Pharmacy in Pharmaceutics, carried out in Department of Pharmaceutics, **Padmavathi College of Pharmacy and Research Institute** under my guidance and supervision.

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LIST OF ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
ATI	Angiotensin I
ATII	Angiotensin II
mEq/L	Milli equivalents per litre
FDA	Food And Drug Administration
SEM	Scanning Electron Microscopy
PBS	Phosphate Buffer Saline
NF	National Formulary
Mg	Milligram
MS	Microspheres
FTIR	Fourier Transform Infrared Spectroscopy
RH	Relative Humidity
GI	Gastro Intestinal
PVP	Poly Vinyl Pyrrolidone
PVA	Poly Vinyl Alcohol
PMMA	Poly Methyl Methacrylate
KV	Kilo Volts
gm	Gram
GRAS	Generally Regarded As Safe
USP	United States Pharmacopoeia
IP	Indian Pharmacopoeia
W/O	Oil in Water
°C	Degree celsius
ml	Milli litre
min	Minutes
KCl	Potassium chloride
Mw	Molecular weight
DMA	Dexamethasone Acetate
PLGA	Polylactic-co-glycolic acid
BSA	Bovine Serum Albumin
rpm	Rotations per minute

INTRODUCTION

1.1. MICROSPHERES AS CONTROLLED DRUG DELIVERY SYSTEM

For many decades, medications of an acute disease or a chronic illness has been accomplished by delivering drugs to the patients via various pharmaceutical dosage forms like tablets, capsules, pills, creams, ointments, liquids, aerosols, injectables and suppositories as carriers. To achieve and then to maintain the concentration of drug administered within the therapeutically effective range needed for medication, it is often necessary to take this type of drug delivery systems several times a day. This results in a fluctuated drug level and consequently undesirable toxicity and poor efficiency.

This factor as well as other factors such as repetitive dosing and predictable absorption leads to the concept of controlled drug delivery systems.[1, 2.]

The objectives of controlled release drug delivery include two important aspects namely spatial arrangement and temporal delivery of drug.

Spatial placement relates to targeting a drug to a specific organ or tissue, while temporal delivery refers to controlling the rate of drug delivery to the target tissue.

An approximately designed controlled release drug delivery system can be a major advance towards solving these two problems. It is this reason that the science and technology responsible for development of controlled release pharmaceuticals have been and continue to be the focus of a great deal of attention both industrial and academic laboratories.

A controlled release system includes any drug delivery system that “achieves slow release of the drug over an extended period of time”. If the system can provide some control weather this of a temporal or a spatial nature, in other words, if the system is successful in maintaining predictable and reproducible kinetics in the target tissue or cell, it is considered as a controlled release system.

If the system only extends the duration of release without reproducible kinetics it is considered as a prolong release system.

The objectives in designing a controlled release system are to deliver the drug at a rate necessary to achieve and maintain a constant drug blood level. This rate should be analogous to that achieved by continuous intravenous infusion where a drug is provided to the patient at a rate just equal to its rate of elimination. This complies that the rate of delivery must be independent of the amount of drug remaining in the dosage form and constant over time. That is release from the dosage form should follow zero-order kinetics[3].

The several advantages of a controlled drug delivery system over a conventional dosage forms:

- Improved patient convenience and compliance due to less frequent drug administration.
- Reduction in fluctuation in steady state levels and therefore better control of disease condition and reduced intensity of local or systemic side effects.
- Increased safety margin of high potency drugs due to better control of plasma levels.
- Maximum utilization of drug enabling reduction in total amount of dose administered.
- Reduction in health care costs through improved therapy, shorter treatment period, less frequency of dosing and reduction in personnel time to dispense, administer and monitor patients[4].

1.2. LISINOPRIL DI HYDRATE

Lisinopril is a potent, competitive inhibitor of angiotensin-converting enzyme (ACE) and is used to treat high blood pressure (hypertension), heart failure and to improve survival after heart attack. Lisinopril, one of the few ACE inhibitors that is not a pro-drug, compete with ATI for binding to ACE and inhibits and enzymatic proteolysis of AT-I to AT-II. Lisinopril, a synthetic peptide derivative, is chemically described as (S)-1-[N2-(1-carboxy-3- phenylpropyl)-L-lysyl]-L-proline dihydrate[5]. Lisinopril inhibits angiotensin-converting enzyme (ACE) in human subjects and animals. ACE is a peptidyl dipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II. Angiotensin II also stimulates aldosterone secretion by the adrenal cortex.

The beneficial effects of Lisinopril in hypertension and heart failure appear to result primarily from suppression of the renin-angiotensin-aldosterone system. Inhibition of ACE

results in decreased plasma angiotensin II which leads to decreased vasopressor activity and to decrease aldosterone secretion. The latter decrease may result in a small increase of serum potassium. In hypertensive patients with normal renal function treated with Lisinopril alone for up to 24 weeks, the mean increase in serum potassium was approximately 0.1mEq/L however, approximately 15% of patients had increases greater than 0.5mEq/L and approximately 6% had a decrease greater than 0.5mEq/L. In the same study, patients treated with Lisinopril and hydrochlorothiazide for up to 24 weeks had a mean decrease in serum potassium of 0.1mEq/L; approximately 4% of patients had increases greater than 0.5 mEq/L and approximately 12% had a decrease greater than 0.5mEq/L.

Concomitant administration of Lisinopril and hydrochlorothiazide further reduced blood pressure in Black and non-Black patients and any racial differences in blood pressure response were no longer evident. Administration of Lisinopril to patients with hypertension results in a reduction of both supine and standing blood pressure to about the same extent with no compensatory tachycardia. Symptomatic postural hypotension is usually not observed although it can occur and should be anticipated in volume and/or salt-depleted patients. In most patients studied, onset of antihypertensive activity was seen at one hour after oral administration of an individual dose of Lisinopril, with peak reduction of blood pressure achieved by 6 hours. Although an antihypertensive effect was observed 24 hours after dosing with recommended single daily doses, the effect was more consistent and the mean effect was considerably larger in some studies with doses of 20 mg or more than with lower doses; however, at all doses studied, the mean antihypertensive effect was substantially smaller 24 hours after dosing than it was 6 hours after dosing. In some patients achievement of optimal blood pressure reduction may require two to four weeks of therapy.

Upon multiple dosing, Lisinopril exhibits an effective half-life of accumulation of 12 hours. Impaired renal function decreases elimination of Lisinopril, which is excreted principally through the kidneys, but this decrease becomes clinically important only when the glomerular filtration rate is below 30mL/min.

Lisinopril 20 mg to 80 mg has been compared in patients with mild to moderate hypertension to hydrochlorothiazide 12.5 mg to 50 mg and with atenolol 50 mg to 200 mg; and in patients with moderate to severe hypertension to Metoprolol 100 mg to 200 mg. It was

superior to hydrochlorothiazide in effects on systolic and diastolic pressure in a population that was 3/4 Caucasian. Lisinopril was approximately equivalent to Atenolol and Metoprolol in effects on diastolic blood pressure, and had somewhat greater effects on systolic blood pressure.

In patients with renovascular hypertension Lisinopril has been shown to be well tolerated and effective in controlling blood pressure.

1.3 Microencapsulation

Microencapsulation is a process by which very tiny droplets or particles of liquid or solid material are surrounded or coated with a continuous film of polymeric material. In microencapsulation particle size is ranging from several tenths to 5000 microns[6].

Microencapsulation provides the means of converting liquids to solids, of altering colloidal and surface properties, of providing environmental protection and of controlling the release characteristics or availability of coated materials. Several of these properties can be attained by macro packaging techniques; however, the uniqueness of microencapsulation is the smallness of the coated particles and their subsequent use and adaptation to a wide variety of dosage forms[7].

REASONS FOR MICROENCAPSULATION[8]

- The primary reason for microencapsulation is found to be either for sustained or prolonged drug release.
- This technique has been widely used for masking taste and odor of many drugs to improve patient compliance.
- This technique can be used for converting liquid drugs in a free flowing powder.
- The drugs, which are sensitive to oxygen, moisture or light, can be stabilized by microencapsulation.
- Incompatibility among the drugs can be prevented by microencapsulation.
- Vaporization of many volatile drugs e.g. methyl salicylate and peppermint oil can be prevented by microencapsulation.
- Many drugs have been microencapsulated to reduce toxicity and GI irritation including ferrous sulphate and KCl.

- Alteration in site of absorption can also be achieved by microencapsulation.
- Toxic chemicals such as insecticides may be microencapsulated to reduce the possibility of sensitization of factorial person.
- Bakan and Anderson reported that microencapsulated vitamin A palmitate had enhanced stability[8].

RELEASE MECHANISMS[8]

Mechanisms of drug release from microspheres are

1. Degradation controlled monolithic system: -

The drug is dissolved in matrix and is distributed uniformly throughout. The drug is strongly attached to the matrix and is released on degradation of the matrix. The diffusion of the drug is slow as compared with degradation of the matrix.

2. Diffusion controlled monolithic system: -

Here the active agent is released by diffusion prior to or concurrent with the degradation of the polymer matrix. Rate of release also depend upon where the polymer degrades by homogeneous or heterogeneous mechanism.

3. Diffusion controlled reservoir system: -

Here the active agent is encapsulated by a rate controlling membrane through which the agent diffuses and the membrane erodes only after its delivery is completed. In this case, drug release is unaffected by the degradation of the matrix.

4. Erosion: -

Erosion of the coat due to pH and enzymatic hydrolysis causes drug release with certain coat material like glyceryl mono stearate, beeswax and steryl alcohol etc.

METHODS TO MANUFACTURE MICROSPHERES[9]

Basic Microencapsulation processes can be divided into

Chemical methods

Physico-chemical methods

Mechanical methods

Chemical methods

- Solvent Evaporation
- Interfacial polymerization/cross linking
- In-situ polymerization
- Matrix polymerization
- Polymer-polymer compatibility
- In-liquid drying
- Thermal and ionic gelation in liquid media

Physico-chemical methods

- Ionotropic gelation
- Coacervation phase separation

Mechanical methods

- Pan coating
- Air suspension coating
- Centrifugal extrusion
- Spray drying
- Spray coating
- Spinning disk or rotational suspension separation
- Electrostatic deposition
- Pressure extraction or spraying into solvent extraction bath[11].

Table No.1

Microencapsulation Processes and Their Applicabilities

S.No.	Method name	Applicable material	Particle size (µm)
1	Air suspension	solids	35-5000
2	Coacervatio-phase seperation	Solids&liquids	2-5000
3	Multi orifice centrifugal process	Solids&liquids	1-5000
4	Pan coating	Solids	600-5000
5	Solvent evaporation	Solids&liquids	5-5000
6	Spray drying and congealing	Solids&liquids	600

Ideal characteristics of drug for microencapsulation[5]

Particle size requirement

The lower the molecular weight, faster and complete is the absorption of the drug. The drugs having size 150-600 Daltons they can easily diffuse through the membrane but diffusivity (the ability of drug to diffuse through the membrane) is inversely related to molecular size.

The drug or protein should not be adversely affected by the process.

Reproducibility of release profile and the method

No stability problem

Drugs unstable in GI environment cannot be administered as oral controlled release formulation because of bioavailability problems. E.g.Nitroglycerine.

There should be no toxic product associated with the final product.

Therapeutic range

A candidate drug for controlled delivery system should have a therapeutic range wide enough such that variations in the release rate do not result in a concentration beyond this level.

Therapeutic index

The ratio of maximum safe concentration to the minimum effective concentration of drug is called as therapeutic index. The release rate of drug with narrow therapeutic index should be such that the plasma concentration is attained between the therapeutically safe and effective range. It is necessary because such drugs have toxic concentration nearer to their therapeutic range.

Elimination half life

Smaller the half life larger the amount of drug to be incorporated in the controlled release dosage form. Drugs with $t_{1\backslash 2}$ in the range of 2-4 hours make a good candidates for such a system. E.g.propronalol.

Plasma Concentration-Response relationship

Drugs whose pharmacological activity is independent of its concentration are poor candidates for controlled release systems.

Microencapsulation of pharmaceuticals is undertaken for the following applications[9, 10]:

Microencapsulation has been employed to provide protection to the core material against atmospheric effects. The separation of incompatible substances, for example pharmaceutical eutectics, has been achieved by encapsulation. Toxic chemicals such as insecticides may be microencapsulated to reduce hazards. Also the hygroscopic properties of many core materials such as sodium chloride may be reduced by microencapsulation.

Microencapsulation by Coacervation Phase Separation Technique[8]

Preparation

The core material will be added to the solution. The core material should not react or dissolve in water (maximum solubility 2%) The core material is dispersed in the solution. The particle size will be defined by dispersion parameter, as stirring speed, stirrer shape, surface tension and viscosity. Size range 2μm - 1200μm

Coacervation

• Coacervation starts with a change of the pH value of the dispersion, e.g. by adding organic acids. The result is a reduction of the solubility of the dispersed phases (shell material).

• The shell material (coacervate) starts to precipitate from the solution.

• The shell material forms a continuous coating around the core droplets.

Cooling and hardening phase

• The shell material is cooled down to harden and forms the final capsule.

• Hardening agents like formaldehyde can be added to the process.

• The microcapsules are now stable in the suspension and ready to be dried.

Drying phase

• The suspension is dried in a spray dryer or in a fluidized bed drier.

• Spray Drying is a suitable method for heat sensitive Products.

• The atomized particles assume a spherical shape. The rapid the coating material keeps the core material below 100°C, even if the temperature in the drying chamber is much greater.

• Microencapsulation makes the spray drying process easier for sticky products like fruit pulp or juice, with a high content of invert sugar.

Coacervation-Phase Separation

The general outline of the processes consists of three steps carried out under continuous agitation:

1. Formation of three immiscible chemical phases – A liquid manufacturing vehicle phase, a core material phase, and a coating material phase. To form the three phases, the core material dispersed in a solution of the coating polymer, the solvent for the polymer being the liquid manufacturing vehicle phase. The coating

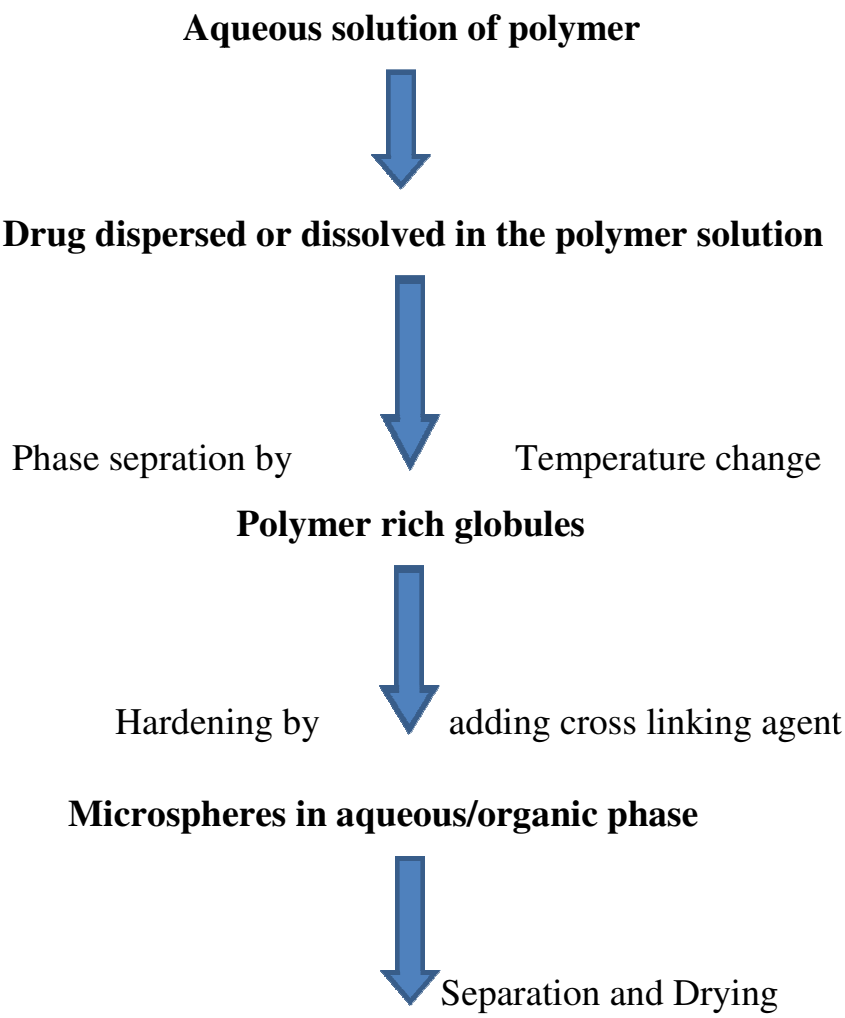
material phase, an immiscible polymer in a liquid state, is formed by utilizing one of the methods of the methods of phase separation-coacervation, i.e., by changing the temperature of the polymer solution; or by adding a salt, nonsolvent, or incompatible polymer to the polymer solution; or by inducing a polymerpolymer interaction.

2. Deposition of the coating – It consists of depositing the liquid polymer coating upon the core material. This is accomplished by controlled, physical mixing of the material in the manufacturing vehicle. Deposition if the liquid polymer coating around the core material occurs if the polymer is adsorbed at the interface formed between the core material and the liquid vehicle phase, and this adsorption phenomenon is a prerequisite to effective coating. The continued deposition of the coating material is promoted by a reduction in the total free interfacial energy of the system, brought about by the decrease of the coating material surface area during coleasance of the liquid polymer droplets.

3. Rigidization of the coating – It involves rigidizing the coating, usually by thermal, cross-linking, or desolvation techniques, to form a self-sustaining microcapsules.

Figure No.1

Preparation of Microspheres by Coacervation Phase Separation Technique



1.4. POLYMERS USED FOR MICROSPHERES[12]

Synthetic Polymers

Non-biodegradable

PMMA

Acrolein

Epoxy polymers

Biodegradable

Lactides and Glycolides copolymers

Polyalkyl cyanoacrylates

Polyanhydrides

Natural polymers

Proteins

Albumins

Gelatin

Collagen

Carbohydrates

Starch agarose

Carrageenan

Chitosan

Chemically modified carbohydrates

Poly (acryl) dextran

Poly (acryl) starch

CLASSIFICATION OF POLYMERS[13]

Water-Soluble Synthetic Polymers

- Poly (acrylic acid) cosmetic, pharmaceuticals, immobilization of Cationic drugs, base for Carbopol polymers.
- Poly (ethylene oxide) Coagulant, flocculent, very high molecular-weight up to a few millions, swelling agent.
- Poly (ethylene glycol) Mw <10,000; liquid (Mw <1000) and wax (Mw >1000), Plasticizer, base for suppositories.
- Poly (vinyl pyrrolidone) used to make betadine (iodine complex of PVP) with less toxicity than iodine, plasma replacement and tablet granulation.
- Poly (vinyl alcohol) Water-soluble packaging, tablet binder, tablet coating.
- Polyacrylamide Gel electrophoresis to separate proteins based on their molecular weights, coagulant, absorbent.

- Poly (isopropyl acrylamide) and poly (cyclopropyl methacrylamide) are thermogelling acrylamide derivatives, its balance of hydrogen bonding, and hydrophobic association changes with temperature.

Cellulose-Based Polymers[13]

- Ethyl cellulose is insoluble but dispersible in water, used in aqueous coating system for sustained release applications.
- Carboxymethyl cellulose is super disintegrant and emulsion stabilizer.
- Hydroxyethyl and hydroxypropyl celluloses are soluble in water and in alcohol, used in tablet coating.
- Hydroxypropyl methyl cellulose is Binder for tablet matrix and tablet coating, gelatin alternative as capsule material.
- Cellulose acetate phthalate generally used in Enteric coating.

Hydrocolloids

- Alginic acid is used in oral and topical pharmaceutical products; thickening and suspending agent in a variety of pastes, creams, and gels, as well as a stabilizing agent for oil-in-water emulsions; binder and disintegrant.
- Gelatin a protein derivative and natural polymer used for controlled release of drug by using suitable cross linking agent.
- Chitosan Cosmetics and controlled drug delivery applications, mucoadhesive dosage forms, rapid release dosage forms.

Water-Insoluble Biodegradable Polymers[13]

- (Lactide-co-glycolide) polymers Microparticle–nanoparticle for protein delivery.

Starch-Based Polymers

- Starch is used as Glidant, a diluent in tablets and capsules, a disintegrant in tablets and capsules, a tablet binder.
- Sodium starch glycolate is super disintegrant for tablets and capsules in oral delivery.

1.5. METHODS TO MANUFACTURE OF GELATIN LOADED MICROSPHERES[8, 10]

Gelatin loaded microspheres can be prepared by using any one of the different following techniques

Single Emulsion Polymerization Technique

Single Emulsion Technique the micro particulate carriers of natural polymers, i.e. those of proteins (e.g. gelatin) and carbohydrates (sodium alginate, chitosan) are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in the non-aqueous medium e.g., oil. In the second step of preparation, cross linking of the dispersed globule is carried out. The cross-linking can be achieved either by means of heat or by using the chemical cross linkers (gluteraldehyde, formaldehyde, calcium chloride, genipin etc.)

Double Emulsion Technique

Double emulsion technique Double emulsion method of microspheres preparation involves the formation of the multiple emulsions or the double emulsion of type w/o/w and is best suited to the water-soluble drugs, peptides, proteins and the vaccines. This method can be used with both the natural as well as the synthetic polymers. The aqueous protein solution is dispersed in a lipophilic organic continuous phase. This protein solution may contain the active constituents.

Spray Drying

Spray drying serves as a microencapsulation technique when an active material is dissolved or suspended in a melt or polymer solution and becomes trapped in the dried particle. The main advantages are the ability to handle labile materials because of the short contact time in the dryer; in addition, the operation is economical. Applying this technique along with the use of supercritical Carbon Dioxide, also sensitive materials like proteins can be encapsulated[8].

Phase Separation Coacervation Technique

Phase Separation Coacervation Technique Phase separation method is specially designed for preparing the reservoir type of the system to encapsulate water soluble drugs e.g. peptides, proteins. Some of the preparations are of matrix type particularly, when the drug is hydrophobic in nature e.g. steroids. In matrix type device, the drug or the protein is soluble in the polymer phase. The process is based on the principle of decreasing the solubility of the polymer in the organic phase to affect the formation of the polymer rich phase called the 'coacervates'.
In this technique the polymer is first dissolved in a suitable solvent and then drug is dispersed by making its aqueous solution, if hydrophilic or dissolved in the polymer solution itself, if hydrophobic. Phase separation is then accomplished by changing the solution conditions by using any of the method mentioned. Salt addition Non-solvent addition addition of in-compatible polymer Change in pH the process is carried out under continuous stirring to control the size of the microparticles[8].

Interfacial Cross Linking

Interfacial cross-linking is derived from interfacial polycondensation, and was developed to avoid the use of toxic diamines, for pharmaceutical or cosmetic applications. In this method, the small bifunctional monomer containing active hydrogen atoms is replaced by a biosourced polymer, like a protein. When the reaction is performed at the interface of an emulsion, the acid chloride reacts with the various functional groups of the protein, leading to the formation of a membrane. The cross-linked protein microcapsules are biocompatible and biodegradable, and the presence of the protein backbone renders the membrane more resistant and elastic than those obtained by interfacial polycondensation[10]. The method is very versatile, and the properties of the microcapsules (size, porosity, degradability, mechanical resistance) can be easily tuned by varying the preparation parameters. A carbohydrate can be added to the protein, for the modulation of particle biodegradability.

Complex Coacervation

Complex coacervation is the spontaneous liquid-liquid phase separation that frequently occurs when solutions of oppositely charged polyelectrolytes are mixed in the same solvent (usually water). It is encountered in biological systems and differs fundamentally from the

polymer-polymer incompatibility mentioned earlier in that one phase contains most of the two polymers whereas the second phase is a dilute polymer solution. Polymer-polymer incompatibility yields two phases with each containing predominately one of the two polymers. For proper encapsulation, it is preferred to have liquid coacervates which can flow around or completely wrap the internal phase particles or droplets.

Solvent Evaporation

The solvent evaporation encapsulation process, depicted is a way of precipitating small polymer particles from an oil-in-water emulsion. The polymer is dissolved in a volatile organic solvent that is immiscible with water. Methylene chloride is a preferred solvent because of its high volatility and its capacity for dissolving a broad range of polymers. A number of solvents that can be used and, it should be noted that, many solvents suitable for this process have a finite degree of water solubility, even though they are normally classified as water-insoluble solvents. Mixed solvents can also be used. The mixtures used so far tend to contain a water-immiscible solvent and a water-miscible solvent (e.g., acetone). The water immiscible solvent is the predominant component of the mixture. Once the desired coating polymer is dissolved in the organic solvent, the drug to be encapsulated is added to this solution. The drug agent may be a solid (crystalline or amorphous) or a nonvolatile liquid. The added drug may completely dissolve in the polymer solution or it may be completely insoluble and simply form a dispersion, suspension, or suspension-emulsion. In the latter case, the solid particles must be micronized so that their mean diameter is much less than the desired mean microsphere size. This is true for any encapsulation technique, and generally, a particle size /microsphere size ratio of 1:10 or less is preferred.

1.6. CHARACTERIZATION OF MICROSPHERES

Particle size, Shape and Morphology

All the microspheres were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle diameters of more than 100 microspheres were measured randomly by optical microscope. Scanning Electron photomicrographs of drug-loaded microspheres were taken. A small amount of microspheres was spread on gold stub. Afterwards, the stub containing the sample was placed in the Scanning electron microscopy (SEM). A Scanning electron photomicrograph was taken at an acceleration voltage of 20KV.

Entrapment Efficiency

The capture efficiency of the microspheres or the percent entrapment can be determined by allowing washed microspheres to lyse. The lysate is then subjected to the determination of active constituents as per monograph requirement. The percent encapsulation efficiency is calculated using following equation

% Entrapment = Actual content/Theoretical content x 100.

Scanning Electron Microscopy (SEM)

The surface morphology of blank microspheres, drug loaded microspheres and microspheres collected after dissolution studies were examined by a Scanning electron microscope (Hitachi, S-3600N, Japan). The samples were fixed on brass sub using double-sided tape and then gold- coated in vacuum by a sputter coater. The SEM pictures were then taken at an excitation voltage of 15 KV[14].

Swelling Index[15]

Swelling index was determined by measuring the extent of swelling of microspheres in the given buffer. To ensure the complete equilibrium, exactly weighed amount of microspheres were allowed to swell in given buffer. The excess surface adhered liquid drops were removed by blotting and the swollen microspheres were weighed by using microbalance. The hydrogel microspheres then dried in an oven at 60°C for 5 h until there was no change in the

dried mass of sample. The swelling index of the microsphere was calculated by using the formula

Swelling index= (mass of swollen microspheres - mass of dry microspheres/mass of dried microspheres) 100.

In Vitro Drug Release

To carry out *In Vitro* drug release, accurately weighed 50 mg of loaded microspheres were dispersed in dissolution fluid in a beaker and maintained at 37±2°C under continuous stirring at 100 rpm. At selected time intervals 5 ml samples were withdrawn through a hypodermic syringe fitted with a 0.4 μm millipore filter and replaced with the same volume of pre-warmed fresh buffer solution to maintain a constant volume of the receptor compartment. The samples were analyzed spectrophotometrically. The released drug content was determined from the standard calibration curve of given drug.

In Vitro Diffusion Studies

In Vitro diffusion studies were performed using in vitro nasal diffusion cell. The receptor chamber was filled with buffer maintained at 37±2°C. Accurately weighed microspheres equivalent to 10 mg were spread on sheep nasal mucosa. At selected time intervals 0.5 ml of diffusion samples were withdrawn through a hypodermic syringe and replaced with the same volume of prewarmed fresh buffer solution to maintain a constant volume of the receptor compartment. The samples were analyzed spectrophotometrically.

Accelerated Stability Studies

The preparation was divided into 3 sets and stored at 4°C (refrigerator), room temperature and 40°C (thermostatic oven). After 15, 30 and 60 days drug content of all the formulation was determined Spectrophotometrically.

LITERATURE REVIEW

Santanu Chakraborty *et al.,* (2010) prepared microparticles of aceclofenac by gelation technique using a blend of sodium alginate and carbopol 934 as release retardant. The entrapment efficiency was found within the range of 73.6 ± 3.21% to 90.7 ± 2.48%. All the formulations were investigated for various evaluation parameters like particle size, flow behavior, percentage swelling, surface morphology study and in vitro drug release etc. All the formulation showed good flow behavior as compared to the pure drug. SEM study revealed that the spheres were almost spherical in shape with rough outer surface. *In-vitro* drug release study showed that by increasing the polymer concentration the drug release of all the formulation were gradually decreased and the optimized formulation (F7) was able to sustain the drug release for 12 hours[15].

Ying Lu, Guoqing Zhang *et al.,* (2007) prepared Flubiprofen gelatin microspheres (FP-GMS) by an emulsion-congealing method. The particle size of FP-GMS with optimized formulation was 2.5, 12.3µm with a mean size of gelatin microspheres of 7.53µm. The drug loading efficiency was 5.02% (w/w). A total of 96% original FP was remained in the microspheres after being stored under 75% humidity and 37°C for 3 months. These data indicate that the simple emulsion-congealing method can be used to encapsulate water soluble drugs[16].

Anand kumar *et al.,* (2011) prepared controlled release of Lamivudine microspheres by W/O/W multiple emulsion solvent evaporation technique using osmogen like sodium chloride, polymers like ethyl cellulose, cellulose acetate and polyvinyl alcohol as a continuous phase. The prepared microspheres were characterized for the percent drug content, encapsulation efficiency, FTIR, scanning electron microscopy (SEM), In vitro dissolution studies, in vitro kinetic studies and accelerated stability studies[17]. The release kinetics data and characterization studies indicate that drug release from microspheres was diffusion-controlled and that the microspheres were stable.

S. S. Bansode *et al.,* (2010) studied the preparation, properties and uses of individually encapsulated novel small particles, as well as significant improvements to tried-and-tested techniques relevant to micro and nanoparticles and their use in a wide variety of industrial, engineering, pharmaceutical, biotechnology and research applications. Its scope extends beyond

conventional microcapsules to all other small particulate systems such as self assembling structures that involve preparative manipulation. The review covers encapsulation materials, physics of release through the capsule wall and desorption from carrier, techniques of preparation, many uses to which microcapsules are put.

P.V. Kozlov *et al.,* (1983) studied Structural diversity of gelatin chain units determines the specific features of gelatin properties. Most synthetic polymers show no such features that are typical of most biopolymers. The first peculiarity of gelatin common to all biopolymers arises from the presence of both acidic and basic functional groups in the gelatin macromolecules. The second peculiarity of gelatin is its capacity to form a specific triple-stranded helical structure not observed in synthetic polymers. The third peculiarity of gelatin as a biopolymer is its specific interaction with water which is different to that observed with synthetic hydrophilic polymers[18].

Vikas Parashar *et al.,* (2010) prepared Bovine Serum Albumin microspheres in four batches. The emulsion cross-linking method was used for the preparation. The quantity of BSA varies for each formulation. The microspheres were spherical, discrete and compact and size distribution was between 33.28 to 36.25 μm. In vitro studies were carried out at different pH for a period of 18 h and compared with marketed formulation. From all the batches it is concluded that when concentration of polymer increases microspheres shows more controlled and prolonged release[19].

Satish Balakrishna Bhise *et al.,* (2005) developed RIF's loaded porous microspheres as a controlled release dosage form. Eudragit based porous microspheres of RIF were prepared by emulsion solvent diffusion method. Prepared porous microspheres were evaluated for its entrapment efficacy, morphology, thermal behavior, crystalline nature, in-vitro drug release and stability in simulated gastric fluid[20]. In vitro drug release studies indicated that drug to polymer ratio of 2:1 showed more than 85% drug release over the period of 3hrs.

Hongyao Zhang *et al.,* (2006) developed polylactic-co-glycolic acid (PLGA) microspheres for continuous delivery of dexamethasone acetate (DMA) for a one-month period. Methods: The microspheres were prepared using an oil-in-water emulsion technique. The release of DMA from optimized PLGA microspheres showed a biphasic pattern. DMA was released immediately during the initial phase then, following this initial burst release, the microsphere system then released the drug in a continuous fashion[21].

Sunil K. Jain *et al.,* (2004) studied microsphere dosage forms have gained increasing importance as oral controlled drug delivery systems. These systems present several advantages in comparison to unit dosage forms such as more predictable gastric emptying and less local irritation. Microsphere systems also minimize the possible intestinal retention of undigested polymer materials in chronic dosing. The Eudragits are a family of polymers based on acrylic and methacrylic acids suitable for use in orally administered drug delivery systems[22].

Kishore Narra *et al.,* (2009) prepared the rifampicin loaded sodium alginate beads by ionic gelation method. In this method, various formulation variables such as the addition of PVA to the drug-alginate mixture and addition of PVP to cross linking solution were studied. PVA and PVP can be used in the formulation to improve in-vitro characteristics of rifampicin loaded alginate beads[23].

Sanat Kumar Basu *et al.,* (2009) prepared Microspheres (MS) of Ketorolac Tromethamine (KT) for oral delivery were prepared by complex coacervation (method-1) and simple coacervation (method-2) methods without the use of chemical cross–linking agent (glutaraldehyde) to avoid the toxic reactions and other undesirable effects of the chemical cross-linking agents. Alternatively, ionotropic gelation was employed by using sodium-tripolyphosphate (Na-TPP) as cross linking agent. Chitosan and gelatin B were used as polymer and copolymer respectively[24].

Bhaskar Mazumder *et al.,* (2009) studied the micro particles mean particle size, percentage yield were decreased significantly with decrease in drug-polymer ratio, surfactant concentration, stirring speed and volume of continuous phase. It was found that in vitro release were decreased significantly with decrease in drug-polymer ratio and stirring speed but increased significantly with increase in surfactant concentration and volume of continuous phase[25].

Chowdary K.P.R *et al.,* (1989) carried out microencapsulation of aspirin, diazepam and nitrofurantoin by calcium alginate. The microparticles were found to be discrete, spherical and free flowing. The release mechanism was found to be of diffusion type and release depended on solubility of the core material in the dissolution fluid[43].

Kakkar A.P *et al.,* (1995) developed and characterized ibuprofen loaded microcapsules with sodium alginate and calcium chloride by ionotropic gelation technique. Spherical, smooth

surfaced alginate microcapsules of ibuprofen were obtained by this method. The preparation was based on dispersion of sodium alginate-ibuprofen matrix in liquid paraffin followed by coating process by calcium chloride[44].

Prasant K Rout *et al.,* (2009) describes preparation of microspheres by solvent evaporation and W/O emulsion solvent evaporation methods followed by in vitro characterization of microspheres to evaluate the effect of method of preparation on physical properties and drug release profile of microspheres. The mean geometric particle size of microspheres prepared by solvent evaporation method was found in the ranges of 40-50 μm and the microspheres prepared by W/O emulsion solvent evaporation method was found in a ranges of 126-150μm, respectively[26].

Alf Lamprecht, Ulrich Schafer *et al.,* (2000) demonstrated the potential of confocal laser scanning microscopy as a characterization tool for different types of microparticles which are prepared by various methods including complex coacervation ,spray drying, double emulsion solvent evaporation technique and ionotropic gelation technique[45].

LakshmanaPrabu S *et al.,* (2009) studied that drug:polymer ratio had a considerable effect on the entrapment efficiency, however particle size distribution of microspheres was more dependent on the volume of dichloromethane and polyvinyl alcohol concentration rather than on the drug: polymer ratio. Drug, polymer concentrations were varied to obtain optimum release profile for sustaining the action of the drug[27].

AIM AND OBJECTIVE

AIM

The main aim of present work is to develop Lisinopril dihydrate loaded Gelatin microspheres by using *Coacervation phase separation technique***.** Oral bioavailability of Lisinopril dihydrate is 25-28%. Dose of Lisinopril is 10-80mg per day, thus conventional dosage form has several draw backs, so the main aim behind this work is to retard the release of drug at controlled rates throughout the day and to improve bioavailability as well as patient compliance by using gelatin and other polymer mixtures and to reduce dosage frequency.

OBJECTIVES

Lisinopril dihydrate is an angiotensin-converting enzyme used in the treatment of hypertension, myocardial infarction, heart failure. It is used in dose of 10-80 mg twice daily.

The present study "Lisinopril microparticles by coacervation phase separation technique" was meticulously designed to improve the therapeutic efficacy and to prolong its release.

The following experimental protocol was therefore designed to allow systemic approach to the study:

1) Compatibility study- compatibility with various polymers by using IR
2) Preparation of standard curve for Lisinopril dihydrate in phosphate buffer (pH 7.4)
3) To prepare microparticles of Lisinopril dihydrate using polymers gelatin, carbopol and sodium alginate by using coacervation phase separation technique.
4) The following evaluation parameters were carried out based on laboratory experiments:
   a) Drug entrapment efficiency
   b) Flow property of prepared microparicles
   c) In-vitro dissolution studies by using dissolution tester USP (XXIII) basket method release.
   d) Analysis by scanning electron microscopy (SEM)
   e) Particle size analysis by optical microscope method.

The objectives of proposed study are as follows:

- To overcome the rapid elimination of drug and to develop the oral controlled drug delivery system.
- To increase the biological half life of the drug or to maintain the constant drug concentration in the body.

The gelatin polymer was selected to prepare microspheres due to its simplicity, low cost and its high entrapment rates achieved with poorly water soluble drugs.

PLAN OF WORK

Present work was carried out to design and evaluate the Lisinopril dihydrate loaded gelatin microspheres.

1) **Selection of drug**
2) **Literature review**
3) **Preformulation studies**

a) Identification of drug

- ✓ physical appearance
- ✓ melting point
- ✓ FT-IR spectra

b) Solubility studies

c) Partition coefficient

d) FT-IR study for drug excipient interaction

e) Determination of absorption maxima (λ max)

4) **Preparation of standard curve in phosphate buffer pH 7.4**
5) **Preparation of gelatin loaded microspheres**
6) **Characterization of prepared gelatin microspheres**
   - Yield of production
   - percent entrapment efficiency
   - particle size analysis
   - surface morphology by scanning electron microscopy (SEM)
7) **Characterization of prepared gelatin microspheres of Lisinopril dihydrate**

   a) Angle of repose

   b) Bulk density

   c) *In-vitro* dissolution studies in phosphate buffer 7.4 pH buffer

d) Interpretation of drug release mechanism by kinetic models

- Zero-order
- First order
- Higuchi model
- Korsmeyer-peppas model.

MATERIALS AND METHODS

The following materials were used as supplied by the manufacturers without further purification:

Table No.2

5.1. Materials Used For Formulations

S. No	Ingredients	Supplied by
1.	Lisinopril dihydrate	Watson pharmaceuticals, Hyderabad.
2.	Gelatin B	Supra pharmaceuticals Ltd. Hyderabad.
3.	Carbopol 934P NF	Supra pharmaceuticals Ltd. Hyderabad.
4.	Sodium alginate	Supra pharmaceuticals Ltd. Hyderabad.
5.	Seasame oil	Yeluri formulations, Hyderabad.
6.	Formaldehyde	S.D Fine chem. Ltd, Mumbai.
7.	Propylene glycol	S.D Fine chem. Ltd, Mumbai.

Table No.3

Equipments Used

S.No.	Equipments	Manufacturer
1.	Remi stirrer No.1A323	Titan Scales Ltd.
2.	Electronic Weighing Balance	Electro labs, India
3.	UV-Visible spectro photometer	Shimadzu
4.	Dissolution test apparatus	USP Rotating basket dissolution.
5.	Scanning electron microscope	JSM 35 CF, JEOL, Japan
6.	FT-IR spectrometer	Shimadzu
7.	Hot air oven	Spectra labs, India

5.2 DRUG PROFILE

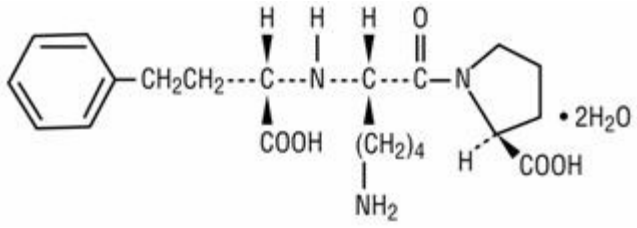
Lisinopril Dihydrate

Lisinopril dihydrate is a lysine derivative, an oral angiotensin-converting enzyme (ACE) inhibitor.

Chemical Name (S)-1-(N2-(1-Carboxy-3-phenylpropyl)-L-lysyl)-L-proline dihydrate

Molecular Weight 441.52

Structural Formula



Category Antihypertensive

Appearance white to off-white, crystalline powder

Solubility It is soluble in water, sparingly soluble in methanol, and practically insoluble in ethanol.

Melting Point Around 160℃

Storage Stored in air tight and light resistant container.

Pharmacokinetics

Following oral administration of Lisinopril, peak serum concentrations of Lisinopril occur within about 7 hours. Lisinopril does not appear to be bound to other serum proteins. Lisinopril does not undergo metabolism and is excreted unchanged entirely in the urine. Declining serum concentrations exhibit a prolonged terminal phase which does not contribute to drug accumulation. This terminal phase probably represents saturable binding to ACE and is not proportional to dose. Systemic bioavailability of Lisinopril dihydrate is approximately 25%[28].

- DOSE : 5-80mg daily
- ONSET OF ACTION : 1Hour
- PEAK PLASMA CONCENTRATION : 6 Hours
- BIOAVAILABILITY : 25%-28%
- EXCRETION : Unchanged in urine
- HALF LIFE ELIMINATION : 12 HOURS on multiple dosing.
- PROTEIN BINDING : Doesn't bound to serum proteins
- VOLUME OF DISTRIBUTION : 28ML/MIN

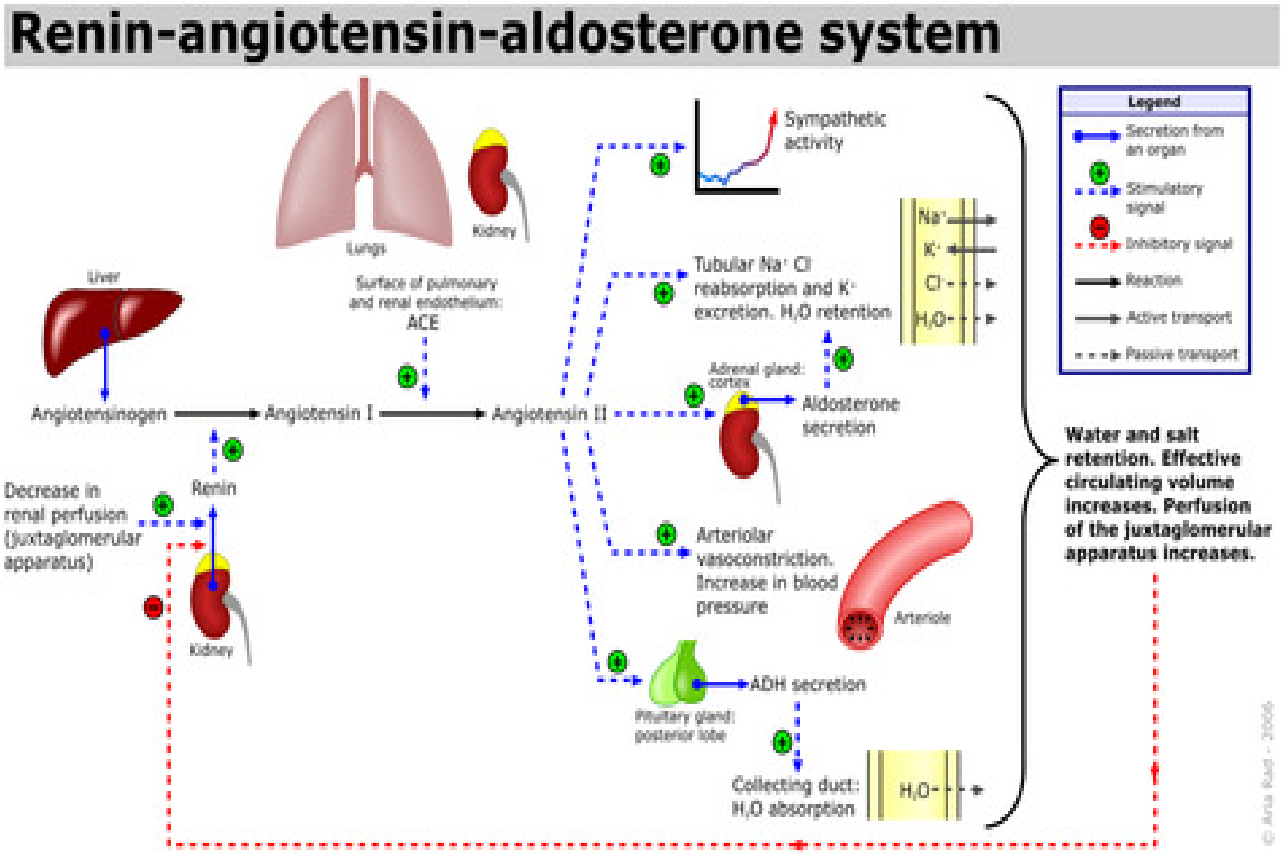
- **Mechanism of Action**

The mechanism through which Lisinopril lowers blood pressure is believed to be primarily suppression of the renin-angiotensin-aldosterone system, Lisinopril is antihypertensive even in patients with low-renin hypertension. Lisinopril inhibits angiotensin-converting enzyme (ACE) in human subjects and animals. ACE is a peptidyldipeptidase that catalyzes the conversion of angiotensin I to the vasoconstrictor substance, angiotensin II. Angiotensin II also stimulates aldosterone secretion by the adrenal cortex. The beneficial effects of Lisinopril in hypertension and heart failure appear to result primarily from suppression of the renin-angiotensin-aldosterone system. Inhibition of ACE results in decreased plasma angiotensin II which leads to decreased vasopressor activity and to decrease aldosterone secretion[28]. The latter decrease may result in a small increase of serum potassium. In hypertensive patients with normal

renal function treated with Lisinopril alone for up to 24 weeks, the mean increase in serum potassium was approximately 0.1mEq/L[29].

Fig.No.2

Mechanism of Action of ACE Inhibitors on Renin-angiotensin System



SIDE EFFECTS

- First doses of Lisinopril can cause dizziness due to a drop in blood pressure.
- Lisinopril can cause nausea, headaches, anxiety, insomnia, drowsiness, nasal congestion, and sexual dysfunction.
- Like all ACE inhibitors, Lisinopril may cause a nonproductive cough that resolves when the drug is discontinued.
- Lisinopril should be stopped if there are symptoms or signs of an allergic reaction including feelings of swelling of the face, lips, tongue or throat. Severe allergic reactions (anaphylaxis) and hives occasionally occur. (Ref FDA).
- Rarely, Lisinopril may cause a decrease in red blood cells (anemia), white blood cells (leukopenia), and platelets (thrombocytopenia).

CONTRAINDICATIONS

Pregnancy: Lisinopril should not be taken during pregnancy because fetuses and neonates have died when Lisinopril was administered during pregnancy[29].

Nursing Mothers: It is not known whether Lisinopril is excreted in breast milk.

5.3 EXCIPIENT PROFILE

5.3.1. Gelatin Type B

Gelatin type B, with isotonic point of 4.8 to 5.2, is the result of an alkaline pretreatment of the collagen. Gelatin is a heterogeneous mixture of single or multi-stranded polypeptides, each with extended left-handed proline helix conformations and containing between 50 - 1000 amino acids. Gelatin is produced by partial hydrolysis of collagen extracted from the boiled bones, connective tissues, organs and some intestines of animals such as domesticated cattle, and pigs[32]. Type A gelatin (dry and ash free) contains 18.5 % nitrogen, but due to the loss of amide groups, Type B gelatin contains only about 18% of nitrogen.

Gelatin melts to a liquid when heated and solidifies when cooled again. Together with water, it forms a semi-solid colloid gel. Gelatin forms a solution of high viscosity in water, which sets to a gel on cooling, and its chemical composition is, in many respects, closely similar to that of its parent collagen.

Gelatin is an amphoteric protein with isotonic point between 5 and 9 depending on raw material and method of manufacture. Like its parent protein, collagen, it is unique in that it contains 14% hydroxyproline, 16 % proline and 26 % glycine.

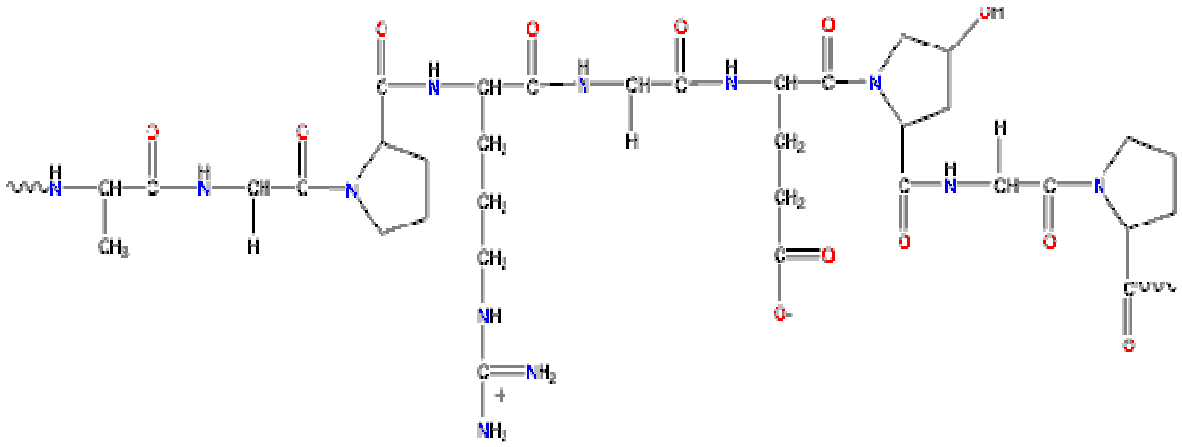
The cross-linking of gelatin with aldehydes is being used to extend the uses of gelatin. In particular, treatment of gelatin films with glutaraldehyde is receiving considerable study in order to improve their thermal resistance, decrease their solubility in water as well as to improve their mechanical properties.

Solution pH (1%) is usually about pH 5 but can vary considerably. At this pH the viscosity of Type B gelatin is minimal and the gel strength is maximal, hence from the manufacturers' point of view it is advantageous to manufacture gelatin at this pH[30].

The most important attribute of gelatin is its gel strength and when determined by the standard method, is called the Bloom Strength. This is the force in grams required to press a 12.5 mm diameter plunger 4 mm into 112 g of a standard $6^2/_3$% w/v gelatin gel at 10°C[30]. Several

penetrometer type instruments have been adapted to determine Bloom Strength. In the present work the bloom strength of gelatin B used was 250.

Structure of gelatin



Empirical formula of gelatin

Gelatin contains many glycine (almost 1 in 3 residues, arranged every third residue), proline and 4-hydroxyproline residues. A typical structure is -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-.

Synonyms

Gelatine, Jelly, gel.

Solubility

The solubility of the gelatin is determined by the method of manufacture[30]. The rate of solubility is affected by factors such as temperature, concentration and particle size. Gelatin is insoluble in cold water but swells and softens when immersed in it, gradually absorbing 5-10 times water to its weight. Soluble in hot water, 6N acetic acid. Insoluble in alcohol, ether and other organic solvents. Gelatin is also soluble in most polar solvents.

Functional categories

USP: Suspending agent, viscosity increasing agent, gelling agent**,** stabilizer[30].

IP: gelling agent , thickener , film former , protective colloid , adhesive agent , stabilizer , emulsifier.

Others: Foaming/whipping agent, beverage fining agent, gelling agent in food and cosmetic manufacturing.

Method of manufacture

Gelatin is made from by-products of the meat and leather industry. Gelatin is derived mainly from pork skins, pork and cattle bones, or split cattle hides. The raw materials are prepared by different curing, acid, and alkali processes which are employed to extract the dried collagen hydrolysate[30]. These processes may take up to several weeks, and differences in such processes have great effects on the properties of the final gelatin products.

The manufacturing processes of gelatin consist of three main stages:

1. Pretreatments to make the raw materials ready for the main extraction step and to remove impurities which may have negative effects on physiochemical properties of the final gelatin product,
2. The main extraction step, which is usually done with hot water or dilute acid solutions as a multi-stage extraction to hydrolyze collagen into gelatin, and finally,
3. The refining and recovering treatments including filtration, clarification, evaporation, sterilization, drying, rutting, grinding, and sifting to remove the water from the gelatin solution, to blend the gelatin extracted, and to obtain dried, blended and ground final product.

Stability and storage conditions

Dry gelatin has an almost infinite shelf life as long as the moisture content is such as to ensure that the product is stored below the glass transition temperature[30].

The stability of gelatin in solution depends on temperature and pH. Generally, to minimize loss of gel strength and viscosity with time, the pH of the solution should be in the range 5 to 7 and the temperature should be kept as low as possible, consistent with the avoidance of gelation and the suitability of the solution viscosity to the particular application.

Safety

Gelatin is regarded as a food ingredient rather than an additive and it is Generally Regarded as Safe (GRAS). In 1993 the FDA reiterated the GRAS status of gelatin and stated that there was no objection to the use of gelatin from any source and any country provided that the hide from animals showing signs of neurological disease were excluded and also Specified Raw Materials were excluded from the manufacturing process.

Application in pharmaceuticals

Microencapsulation: Gelatin is amphoteric, i.e. it has both positive and negative charges on the molecule (and no net charge at the isoionic point). Hence, at a pH where the basic side chains do not carry a charge, acid groups for example from gum arabic can react with the basic groups of gelatin to form an insoluble gelatin-arabate complex which can be precipitated around emulsified oil droplets, forming micro-ecapsulated oil. The microcapsules are hardened with formaldehyde or gluteraldehyde before harvesting and drying[31].

Milk – Food stabilizing industry: Gelatin is used as a stabilizer particularly in yoghurt, where the addition of 0.3 - 0.5 % acts to prevent syneresis thus allowing the production of stirred and fruit containing products.

Emulsifying properties: Gelatin gives limited emulsifying and emulsion stabilising properties used in the manufacture of toffees and water in oil emulsions like low fat margarine[31].

Texturising properties: Gelatin is used in dried soups to provide the appropriate mouth feel (viscosity) to the final product.

Film forming properties: Gelatin's film forming properties are used in the manufacture of both hard and soft (pharmaceutical) capsules.

Adhesive properties: An example of this use of gelatin is in pharmaceutical or confectionery tableting and in liquorices' all-sorts where it can be used to join the layers.

Gelling properties: In the manufacture of these products gelatin is combined with sugar and glucose syrups.

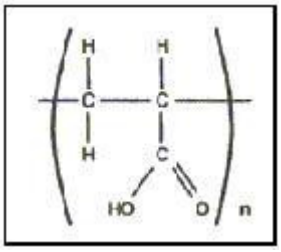
Review of past work on gelatin

BSA-loaded gelatin microspheres were prepared they are with a high loading efficacy and a particle size from 1 to 10*μm. In vitro* degradation and drug release studies were performed in order to evaluate the potential of gelatin microspheres as regulated and sustained release systems. During 4 days, between 80 and 91 % of gelatin microspheres were degraded. The diffusion exponents were superior to 0.5 indicating the anomalous Fickian diffusion, thus suggesting the influence of a polymer relaxation and diffusion through the matrix in BSA release[33].

Microspheres (MS) of Ketorolac Tromethamine (KT) for oral delivery were prepared by complex coacervation (method-1) and simple coacervation (method-2) methods without the use of chemical cross–linking agent (glutaraldehyde) to avoid the toxic reactions and other undesirable effects of the chemical cross-linking agents. Alternatively, ionotropic gelation was employed by using sodium-tripolyphosphate (Na-TPP) as cross linking agent. Chitosan and gelatin B were used as polymer and copolymer respectively[34].

5.3.2. Carbopol 934P NF (*"carboxy polymethylene"*)

Structural formula



Empirical formula

$(C_3H_4O_2)_n$

Description

It is fluffy, white, dry powder (100% effective). The carboxyl groups provided by the acrylic acid backbone of the polymer are responsible for many of the product benefits. Carbopol 934P NF is cross-linked with allyl sucrose and is polymerized in solvent benzene.

Method of manufacture

Carbomers are synthetic, high-molecular-weight, cross linked polymers of acrylic acid. These acrylic acid polymers are cross linked with allyl sucrose or allyl pentaerythritol. The polymerization solvent used previously was benzene; however, Carbopol 934P NF is cross-linked with allyl sucrose and is polymerized in solvent benzene[35].

Grades

Various grades of carbopol are available like Carbopol 71G, 971 P, 974 P, 940NF, 934P NF, 1342 NF, 980NF with different viscosities. For present work we used carbopol 934P NF grade having viscosity 29400 – 39400.

Solubility

Swellable in water, glycerin and after neutralization in ethanol (95%). Carbomers do not dissolve but merely swell to a remarkable extent, since they are three-dimensionally cross linked micro gels. In addition to its hydrophilic nature, its cross-linked structure and it's essentially insolubility in water makes Carbopol a potential candidate for use in controlled release drug delivery systems.

Stability and storage conditions

Carbomers are stable, hygroscopic materials that may be heated at temperatures below 104℃ for up to 2 hours without affecting their thickening efficiency. However, exposure to excessive temperatures can result in discoloration and reduced stability. Dry powder forms of carbomer do not support the growth of molds and fungi. At room temperature, carbomer dispersions maintain their viscosity during storage for prolonged periods.

Safety

Carbomers are generally regarded as essentially nontoxic and nonirritant materials. There is no evidence of systemic absorption of carbomer polymers following oral administration. Acute oral toxicity studies in animals indicate that carbomer 934P NF has a low oral toxicity.

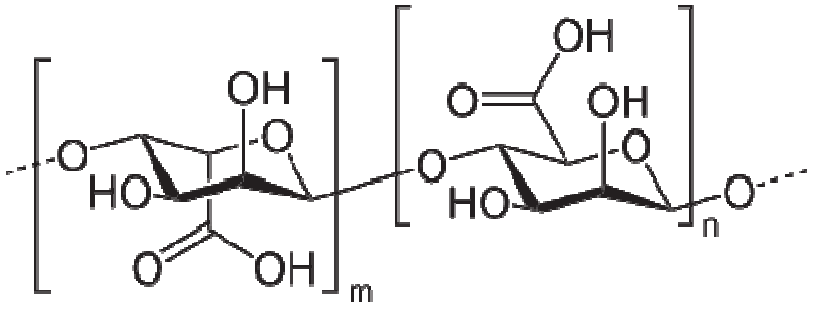
Applications

The readily water swellable Carbopol polymers are used in a diverse range of pharmaceutical applications to provide:

- Controlled release in tablets.
- Bioadhesion in buccal, ophthalmic, intestinal, nasal, vaginal and rectal applications.
- Thickening agent in topical, lotions, creams and gels, oral suspensions and transdermal gel reservoirs.
- Permanent suspensions of insoluble ingredients in oral suspensions and topicals.
- Emulsifying topical oil-in-water systems permanently, even at elevated temperatures, with essentially no need for irritating surfactants.

5.3.3. Sodium Alginate

Structural formula



Empirical formula

$(CH_7O_6Na)_n$

Description

Sodium alginate occurs as a white or buff powder which is odorless and tasteless. Sodium alginate produces aqueous solutions that forms gel on the addition of small amount of soluble calcium salt.

Grades

Various grades of sodium alginate are available yielding aqueous solutions of varying viscosities within a range of 20-400 centipoises.

Solubility

Sodium alginate is very slowly soluble in water forming a viscous colloidal solution practically it is insoluble in alcohol, chloroform and ether and in hydro alcoholic solutions in which alcohol content is greater than 30% by weight.

Stability and storage conditions

Sodium alginate is hygroscopic; the moisture content at equilibrium is a function of relative humidity. Dry storage stability is excellent when the powder is stored in a well closed container at temperatures of 25℃ or less[36].

Incompatibility

Depending on the concentrations sodium alginate is incompatible with phenols and parabens[37].

Applications of Sodium Alginate

- Food Application
- Pharmaceutical Application
- Dental application
- Cosmetic Application

Food Application

Used as an emulsifier and thickener in areas like food industry. It is also used in dairy products to enhance taste and flavor.

Pharmaceutical Application

Sodium Alginate fibers are used to weave bandages for larger wounds and burns and are used as an additive in treating skin diseases.

Used as suspending and thickening agents aid in the preparation of water miscible pastes, creams and gels[36].

Dental Application

Commonly used by dentists.

5.4 PREFORMULATION STUDIES

Prior to the development of the dosage forms the preformulation study was carried out. Hence infrared spectra of the physical mixture of the drug and the polymers chosen were taken. The infrared spectra of the drug and polymers were also taken.

The application of infra-red spectroscopy lies more in the qualitative identification of substances either in pure form or in the mixtures and as a tool in establishment of the structure. Since IR is related to covalent bonds, the spectra can provide detailed information about the structure of molecular compounds. In order to establish this point, comparisons can be made between the spectrum of the substance and the drug.

The above discussions imply that infra-red data is helpful to confirm the identity of the drug and to detect the interaction of the drug with the carriers.

Preformulation studies give the information need to define the nature of the drug substance and provide a frame work for the drug combination with pharmaceutical excipients in the fabrication of a dosage form.

Physical appearance

Lisinopril dihydrate is a white to off-white, crystalline powder.

Melting Point

Melting point was found around 160^0C with decomposition.

Partition Coefficient

At room temparature (n-Octanol/0.1M pH-7 phosphate buffer) found to be 10.2±0.5.

Determination Of λmax

An absorption maximum of Lisinopril dihydrate was found to be 258nm.

SOLUBILITY STUDIES

The solubility of Lisinopril dihydrate was determined in solvents of different polarities. The solubility of Lisinopril dihydrate is usually determined by the equilibrium solubility method, which employs a saturated solution of Lisinopril dihydrate, obtained by adding an excess amount

of Lisinopril dihydrate in the solvent to promote drug precipitation, and then stirring for 24hrs until equilibrium reached. The mixture was filtered and amount of Lisinopril dihydrate was determined using UV spectrophotometer at 258nm.

Table No.4

Solubility Studies of Lisinopril Dihydrate in Various Solvents

S. No.	Solvents	Observed
1	Distilled Water	Freely soluble
2	PBS-7.4(pH)	Freely soluble
3	Ethanol	Practically Insoluble
4	Acetonitrile	Practically Insoluble

5.5 STANDARD PLOT FOR LISINOPRIL DIHYDRATE:

Standard graph by using phosphate buffer (pH 7.4)

Accurately weighed 10mg of Lisinopril dihydrate was dissolved in 100ml of 7.4 pH buffer solution to form 100mcg/ml stock solution.

From the above stock solution aliquots of 10ml, 20ml, 30ml, 40ml, 50ml were pipetted out into a series of 100ml volumetric flasks and volume was made up to 100ml in order to get a concentration ranging from 10-50mcg/ml.

The absorbance of the resulting solution was then measured at 258nm using UV-Spectrophotometer against respective parent solvent as blank. The standard curve was obtained by plotting absorbance Vs concentration in mcg/ml.

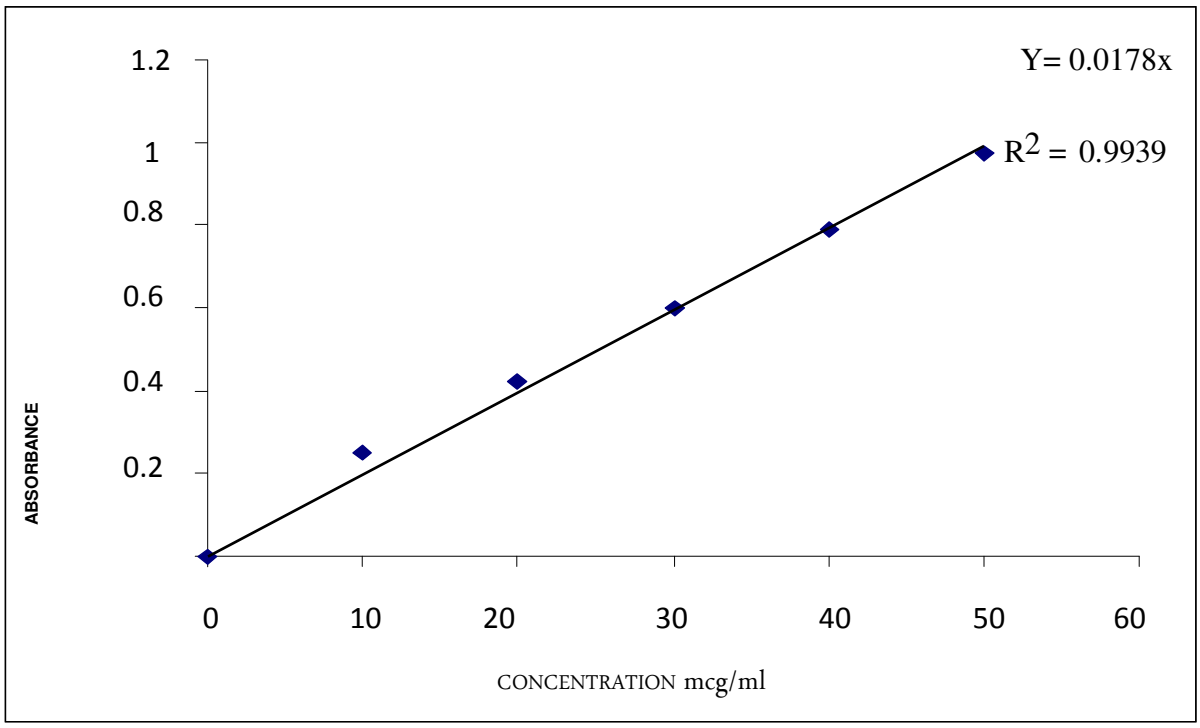
Table No.5

Standard calibration curve of Lisinopril dihydrate

S.No	Concentration (mcg/ml)	Absorbance
1	10	0.220
2	20	0.433
3	30	0.617
4	40	0.789
5	50	0.980

Graph No.1

Calibration curve of Lisinopril dihydrate



Slope= 0.0178

Calibration Curve For Lisinopril Dihydrate in Phosphate Buffer pH 7.4 at 258nm.

FORMULATION AND DEVELOPMENT

Preparation of Microparticles

Gelatin, gelatin-carbopol and gelatin-sodium alginate mixture containing Lisinopril dihydrate Micro spheres were prepared by "*coacervation phase separation technique"* utilizing temperature change. In this method weighed amount of Gelatin was dissolved in 10ml of water which was previously heated to 50˚ C, to this Lisinopril was added and stirred approximately at 300 rpm with the help of magnetic stirrer for 15 mins to get a stable dispersion. The dispersion was poured drop wise into the 100ml of seasome oil which was also previously heated to 50˚ C on a water bath. The mixture was stirred with a help of magnetic stirrer for 2 hrs at 300rpm at room temperature[38].

At the end of 2 hrs cross linking agent formaldehyde 0.5ml was added to the dispersion medium and stirring was continued for next 30 mins. Finally it was kept in refrigerator for 24 hrs to ensure the rigidization of microspheres. Then the microspheres were washed with propylene glycol to wash off excess oil. This Procedure was followed to prepare 6 batches of Lisinopril microspheres with different ratios of gelatin and gelatin-carbopol, gelatin-sodium alginate mixtures. The amount of drug and polymers used were given in Table No.6

Table No.6

Formulation Design of Microparticles

Batch No.	Lisinopril Dihydrate (mg)	Gelatin (gm)	Carbopol (gm)	Sodium alginate (gm)	Formaldehyde (ml)
F1	20	1	-	-	0.5
F2	20	1.5	-	-	0.5
F3	20	0.75	0.25	-	0.5
F4	20	0.50	0.50	-	0.5
F5	20	0.75	-	0.25	0.5
F6	20	0.50	-	0.50	0.5

EVALUATION STUDIES

7.1. Particle size determination[38, 39]

The particle size of a pharmaceutical substance is strictly maintained in order to get optimal biological activity.

Methods to estimate particle size

a. Optical microscopy
b. Sieving method
c. Sedimentation method
d. Elutriation method
e. Centrifugal defractometry
f. Permeability method
g. Light scattering method

Table No.7

Common techniques for measuring fine particles of various sizes

S.No	Technique	Particle size in µm
1	Optical microscopy	1-100 µm
2	Sieving	>50 µm
3	Sedimentation	>1 µm
4	Elutriation	1-50 µm
5	Centrifugal	<50 µm
6	Permeability	>1 µm
7	Light	0.5-50 µm

Particle size analysis

The Particle size analysis was carried out by using optical microscopy. About 200 Micro spheres were selected randomly and their size was determined by using optical microscope fitted with standard micrometer scale. The mean particle size of Micro spheres was given in Table No.8

Table No.8

Mean Particle size of microsphere formulations

S.No	Formulation	Particle size (µm)
1	F1	67.9
2	F2	76.4
3	F3	69.2
4	F4	73.7
5	F5	68.5
6	F6	70.3

Flow properties[38, 39]

Irregular flow of powder from the hopper produces tablets and capsules with no uniform weights. Flow properties depend on particle size, shape, porosity and density of the powder. The flow properties of microspheres were studied by determining various parameters like the angle of repose and bulk density. The angle of repose was determined by the fixed-base cone method. Bulk density is determined using digital bulk density apparatus (Electrolab, India).

7.2. Determination Angle of Repose

The flow characteristics are measured by angle of repose. Improper flow is due to frictional forces between the particles. These forces are quantified by angle of repose. Angle of repose is defined as maximum angle possible between the surface of the pile of the powder and the horizontal plane. The flow of powder and the angle of repose are depicted in the following table.

Definition

Lower the angle of repose the better the flow property. Rough and irregular surface of particles gives higher angle of repose. Decrease in the particle size leads to a higher angle of repose.

$\text{Tan}\Theta = h/r$

$\Theta = \tan^{-} 1\ (h/r)$

Where, h = height of pile

r = radius of base of pile

Θ = angle of repose

Method

A glass funnel is held in place with a clamp on a ring support over a glass plate. The glass plate is placed on a stand. Approximately 100 mg of a particle is transferred into funnel keeping the orifice of the funnel blocked by the lower thumb. As the thumb is removed the particles are emptied from funnel, and the angle of repose is determined by above mentioned formula.

Table No.9

Relation between Angle of Repose and Flow of the Particles

Angle of repose (degrees)	Type of flow
<25	Excellent
25-30	Good
30-40	Passable
>40	Very poor

Table No.10

Angle of Repose of Microparticles

S.No	Formulation	Angle of repose
1	F1	25º 40’
2	F2	26º 28’
3	F3	26 º44’
4	F4	24 º48’
5	F5	25º 34’
6	F6	24º 26’

7.3. Determination of Bulk Density

Bulk density is defined as the weight per unit volume of material. Bulk density is primarily used for powders or pellets. The test can provide a gross measure of particle size and dispersion which can affect material flow consistency and reflect packaging quantity.

Table No.11

Bulk Density of Formulations

Batch no.	Bulk density(g/ml)
F1	0.55
F2	0.56
F3	0.65
F4	0.46
F5	0.53
F6	0.46

7.4. DRUG ENTRAPMENT EFFICIENCY[40]

Drug entrapment efficiency of Lisinopril dihydrate was performed by accurately weighing 20 mg of micro particles and suspended in 20ml of simulated intestinal fluid of pH 7.4± 0.1 and it was kept for 12hrs. Next day it was stirred for 15mins, and subjected for filtration. After suitable dilution, Lisinopril dihydrate content in the filtrate was analyzed spectrophotometrically at 258nm using Shimadzu UV-visible Spectrophotometer.

The absorbance found from the UV-spectrophotometer was plotted on the standard curve to get the concentration of the entrapped drug. Calculating this concentration with the dilution factor we get the percentage drug encapsulated in microparticles.

Encapsulation efficiency was calculated using the following formula

$$\text{Theoretical drug content} = \frac{\text{Amount of drug in bead}}{\text{Amount of bead taken}} \times 100$$

$$\text{Encapsulation efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

Table No.12

Drug entrapment Efficiency of Microparticles

Formulations	Drug Entrapment Efficiency (%)
F1	60.78
F2	65.30
F3	77.86
F4	80.23
F5	82.76
F6	83.24

7.5. Determination of Percentage yield of microspheres

Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formula

$$\% \text{ Yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer}} \times 100$$

Table No.13

Percentage yield of formulations

Formulation	Percentage yield (%)
F_1	82.64
F_2	78.90
F_3	80.95
F_4	88.53
F_5	80.50
F_6	84.16

7.6. SCANNING ELECTRON MICROSCOPY

Procedure

Morphology details of the specimens were determined by using a scanning electron microscope (SEM), Model JSM 35CF, JEOL, Japan. The samples were dried thoroughly in vacuum desiccator before mounting on brass specimen studies. The samples were mounted on specimen studies using double sided adhesive type, and gold palladium alloy of 120Å knees was coated on the sample using sputter coating unit (Model E5 100 Polaron U.K) in Argon ambient of 8-10 Pascal with plasma voltage about 20MA. The sputtering was done for nearly 3mins to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage of about 15KV with load current of about 80 MA.

The condenser lens position was maintained in between 4.4 - 5.1. The objective lens aperture has a diameter of 240 microns and the working distance WD=39mm[41, 42].

Fig No.3 SEM of Formulation F4 under Low Magnification

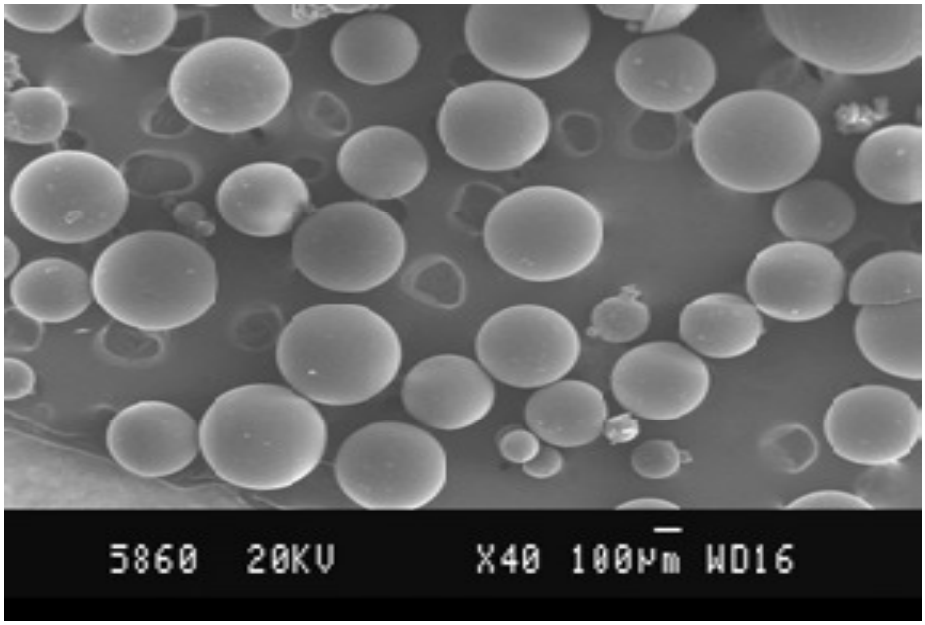
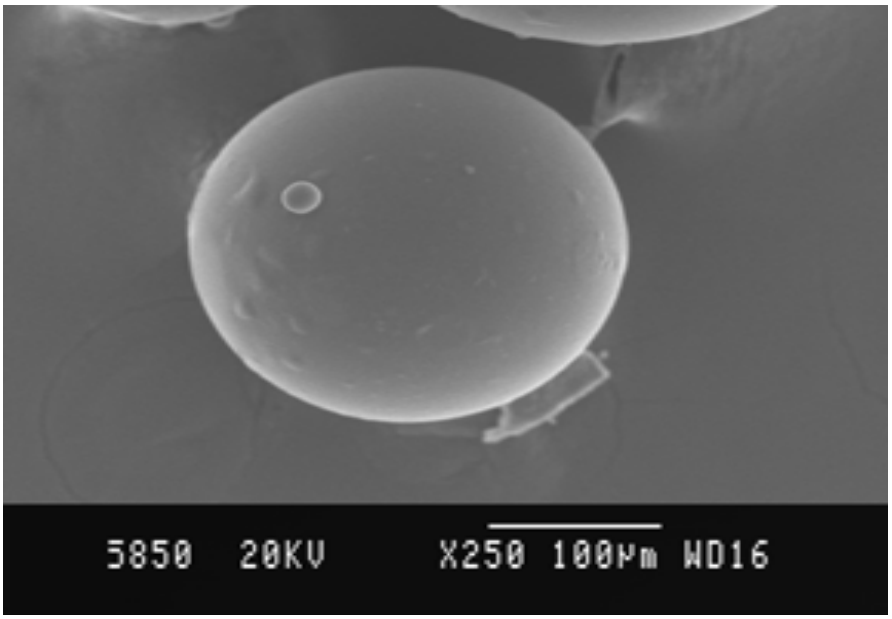


Fig No.4 Surface View of Microsphere of Formulation F4



7.7. FT-IR Spectra of Lisinopril Dihydrate

The FT-IR analysis of the Lisinopril dihydrate was carried out for quantitative compound identification. The FT-IR graph was taken in the range of 4000cm^{-1} – 450cm^{-1} and with the resolution 4cm^{-1}. The FT-IR spectra for pure drug was obtained by KBr pellet method and also was employed to detect chemical interaction between drug and polymers and results were given in below table and graphs.

Table No.14

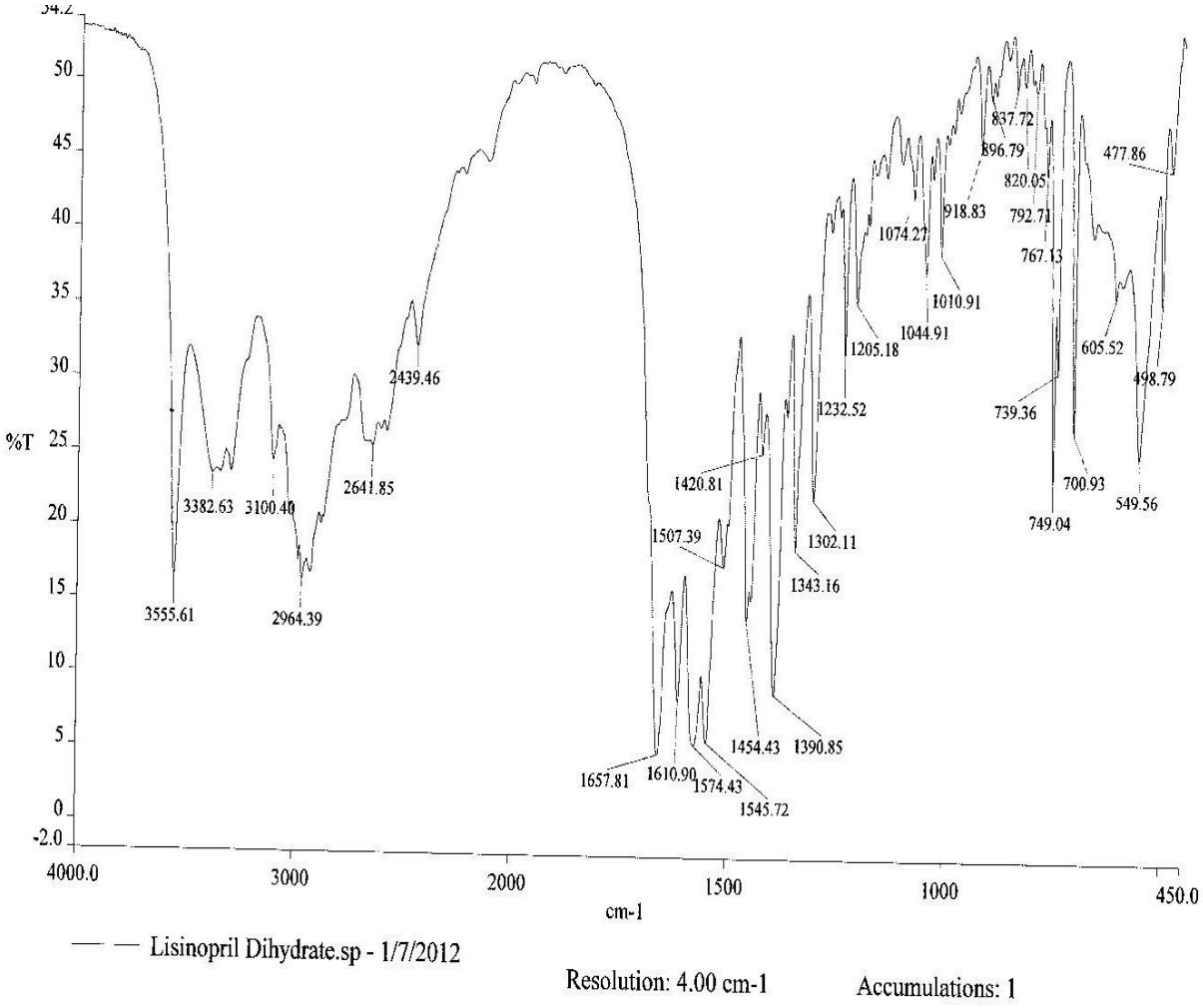
Comparision of IR Spectra of Lisinopril dihydrate and in combination with polymers

S.No.	System	-NH stretch (cm^{-1})	$-NH_2$ stretch (cm^{-1})	-C=N stretch (cm^{-1})	-COOH stretch (cm^{-1})	C=C stretch (cm^{-1})
1	Lisinopril dihydrate (LDH)	3296.32	3555.59	1658.99	1343.12	2964.39
2	LDH + Gelatin	3295.51	3556.59	1658.48	1343.11	2964.02
3	LDH + Carbopol	3295.45	3556.48	1658.90	1343.14	2964.15
4	LDH + Sodium alginate	3296.33	3555.16	1658.95	1343.10	2964.10

LDH - Lisinopril dihydrate.

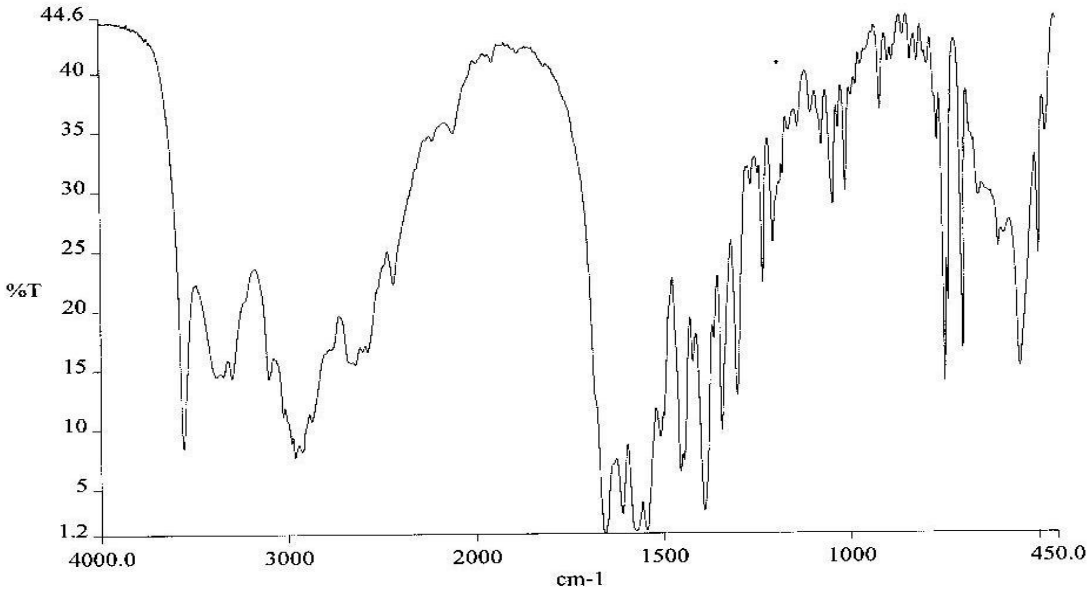
Graph No.2

FT-IR graph of Lisinopril dihydrate (pure drug)



Graph no.3

FT-IR graph of Lisinopril dihydrate + Gelatin



Spectrum Name: Lisinopril Dihydrate+Gelatin.sp

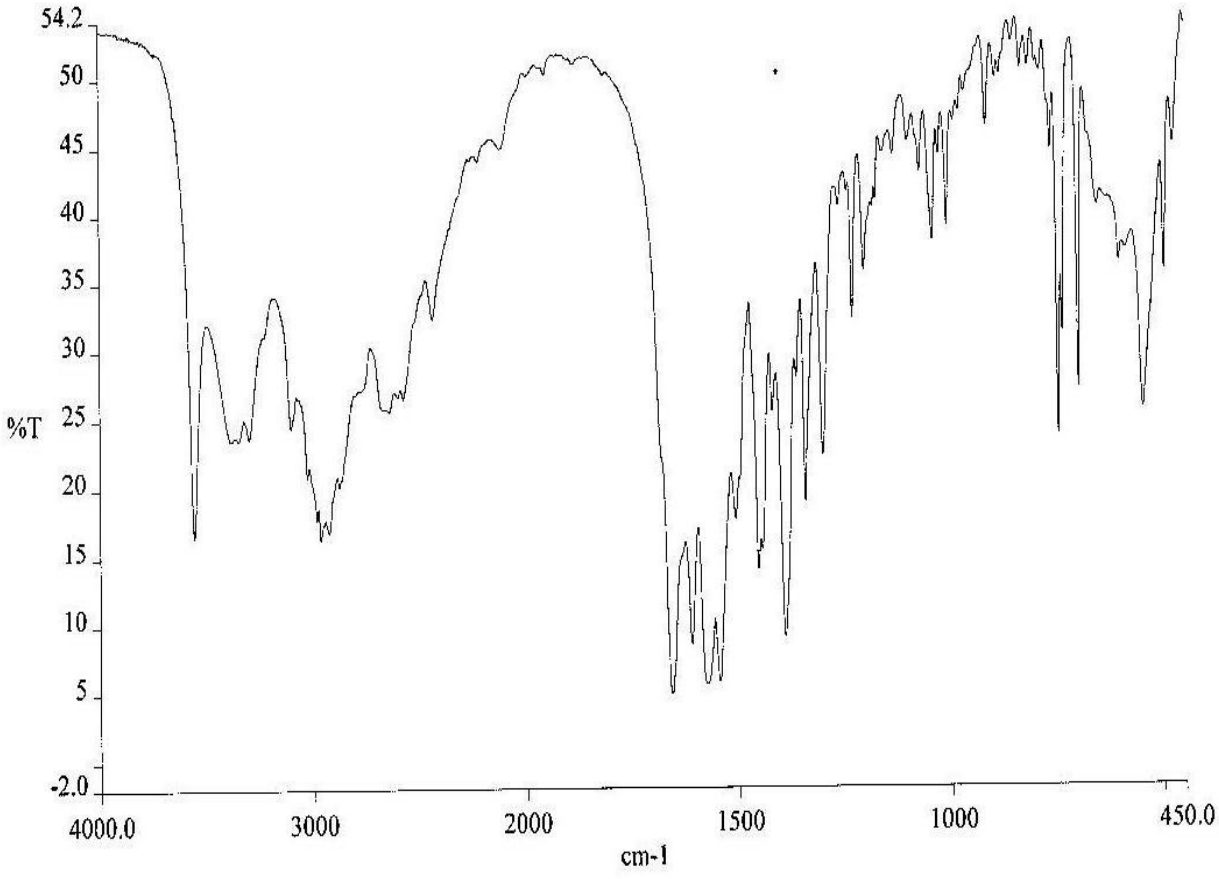
Lisinopril Dihydrate+Gelatin.pk

LISINO~1.SP 3551 4000.00 450.00 1.18 44.61 4.00

REF 4000 44.30 2000 40.77 600
3556.01 8.43 3295.51 14.33 2964.02 7.65 2642.67
1658.48 1.17 1611.02 2.87 1574.51 1.39 1545.73 1
1454.56 6.32 1420.52 15.53 1390.91 3.13 1343.11
1232.48 22.10 1205.05 25.56 1074.36 33.70 1044.94
918.78 36.75 896.70 40.68 837.73 40.85 819.84 4
767.10 34.13 749.03 13.91 739.36 20.57 701.03 1
549.67 15.19 498.77 24.51 478.28 34.79

Graph No.4

FT-IR graph of Lisinopril dihydrate + Carbopol



Spectrum name: Lisinopril dihydrate + carbopol.sp

Lisinopril dihydrate + carbopol.pk

3295.45

3556.48

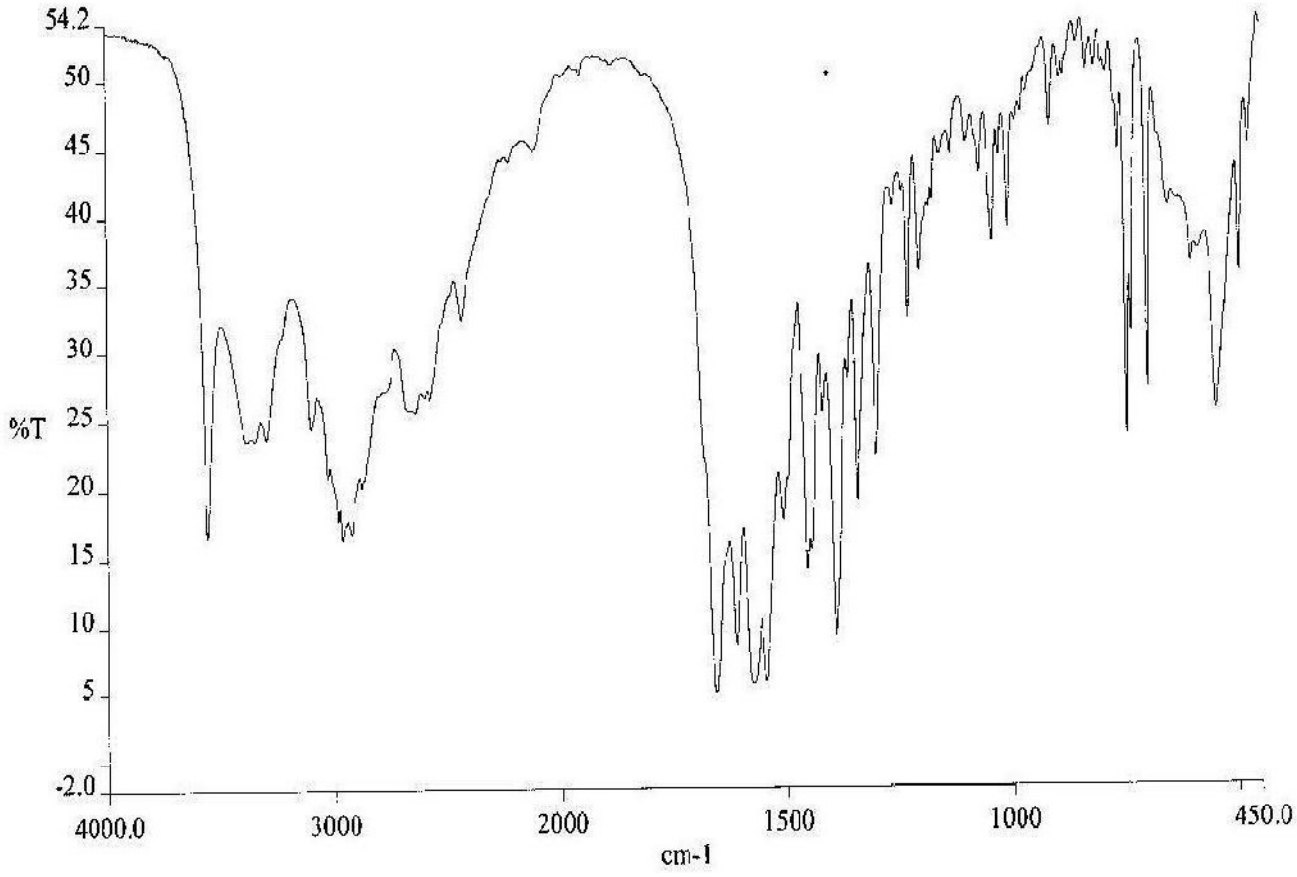
1658.90

1343.14

2964.15

Graph No.5

FT-IR graph of Lisinopril dihydrate + Sodium alginate



Spectrum name: Lisinopril dihydrate+sodium alginate.sp

Lisinopril dihydrate+sodium alginate.pk

3296.33

3555.16

1658.95

1343.10

2964.10

7.8. *IN-VITRO* DISSOLUTION STUDIES

A drug is expected to release from the solid dosage forms (granules, tablets, microspheres, capsules etc.) and immediately go into molecular solution. This process is called as dissolution.

Drug release studies

The method specified in USP for the drug release study was followed.

Apparatus

USP XXIII dissolution test apparatus employing the round bottom dissolution vessel and rotating basket assembly.

Buffer stage

900ml of pH 7.4 and intestinal fluid Ts (phosphate buffer)

Time

For every 2 hrs interval samples were taken and this was continued till 18 hrs.

Procedure

In-vitro release profile of the microparticles was evaluated using rotating basket dissolution apparatus. 900ml of phosphate buffer (pH 7.4) maintained at 37 ± 0.5° C was used as dissolution medium, and the basket was rotated at a constant speed of 75 rpm. Accurately weighed amount of microparticles equivalent to 20 mg of drug were placed in baskets.

Aliquots of samples were withdrawn at the interval of 2 hrs for 18 hrs in phosphate buffer pH 7.4. The samples withdrawn were filtered, diluted suitably and analyzed at 258nm spectrophotometrically for drug release.

7.9. Kinetics of drug release

The data obtained from the in-vitro dissolution studies was subjected for kinetic treatment to obtain the order of release and best fit model for the formulations.

Table No.15

***In-Vitro* Dissolution Profile for Formulation F1**

Time (Hrs)	Absorbance	Concentration	Amount in 900ml (mg)	Cumulative % Drug released
2	0.022	1.25	2.2	11.3
4	0.049	2.77	5.0	25
6	0.099	5.61	10.1	50.5
8	0.113	6.36	11.4	57.3
10	0.125	7.04	12.6	63.4
12	0.136	7.67	13.8	69.1
14	0.146	8.24	14.8	74.2
16	0.157	8.85	15.9	79.7
18	0.168	9.44	17	85

Graph No.6

Dissolution Profile of Formulation F1

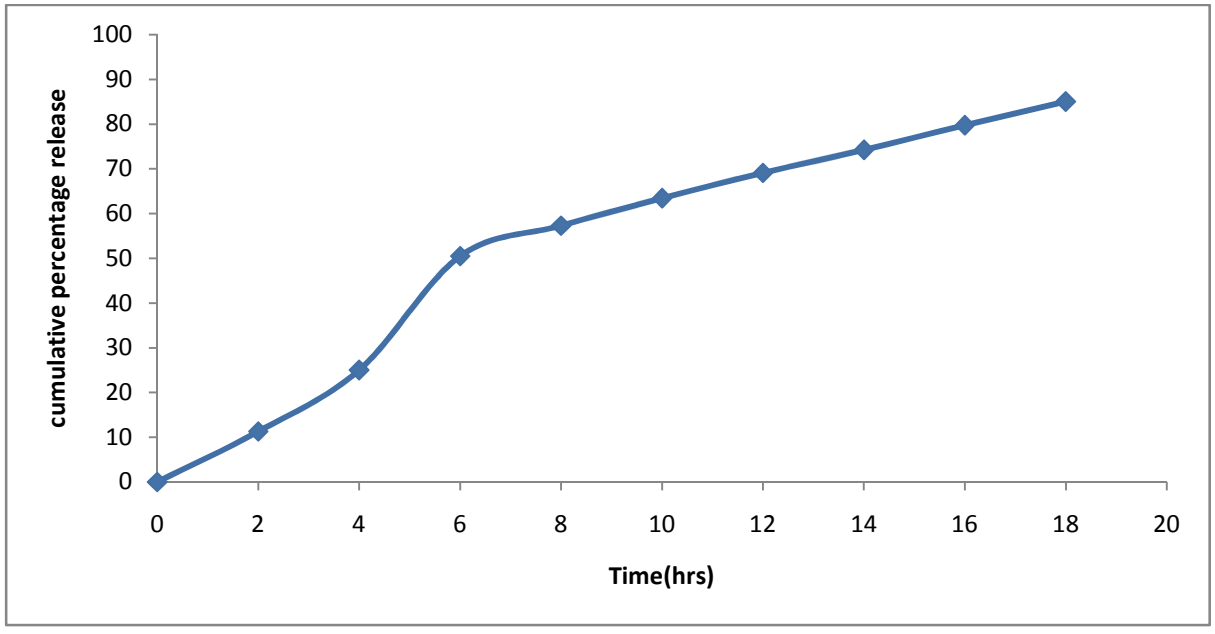


Table No.16

***In-vitro* Dissolution Profile For Formulation F2**

Time (Hrs)	Absorbance	Concentration	Amount in 900ml (mg)	Cumulative % Drug released
2	0.027	1.53	2.76	13.8
4	0.042	2.36	4.26	21.3
6	0.080	4.53	8.16	40.8
8	0.011	6.18	11.1	55.7
10	0.123	6.92	12.4	62.3
12	0.130	7.35	13.6	68.2
14	0.141	7.97	14.3	71.8
16	0.150	8.43	15.1	75.9
18	0.157	8.82	15.8	79.4

Graph No.7

Dissolution Profile of Formulation F2

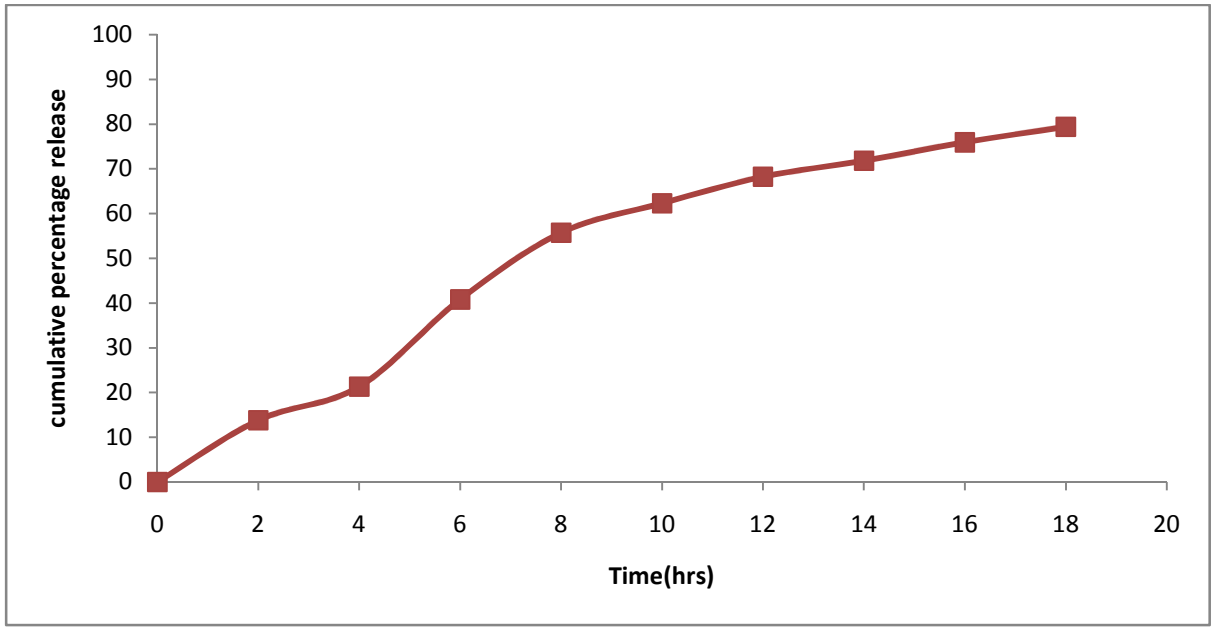


Table No.17

***In-vitro* Dissolution Profile For Formulation F3**

Time (Hrs)	Absorbance	Concentration	Amount in 900ml (mg)	Cumulative % Drug released
2	0.022	1.25	2.26	11.3
4	0.039	2.22	4.0	20
6	0.089	5.00	9.0	45
8	0.119	6.72	12.1	60.8
10	0.124	7.00	12.6	63.1
12	0.131	7.4	13.3	66.7
14	0.137	7.7	13.8	69.3
16	0.139	7.8	14.1	70.5
18	0.143	8.0	14.5	72.8

Graph No.8

Dissolution Profile of Formulation F3

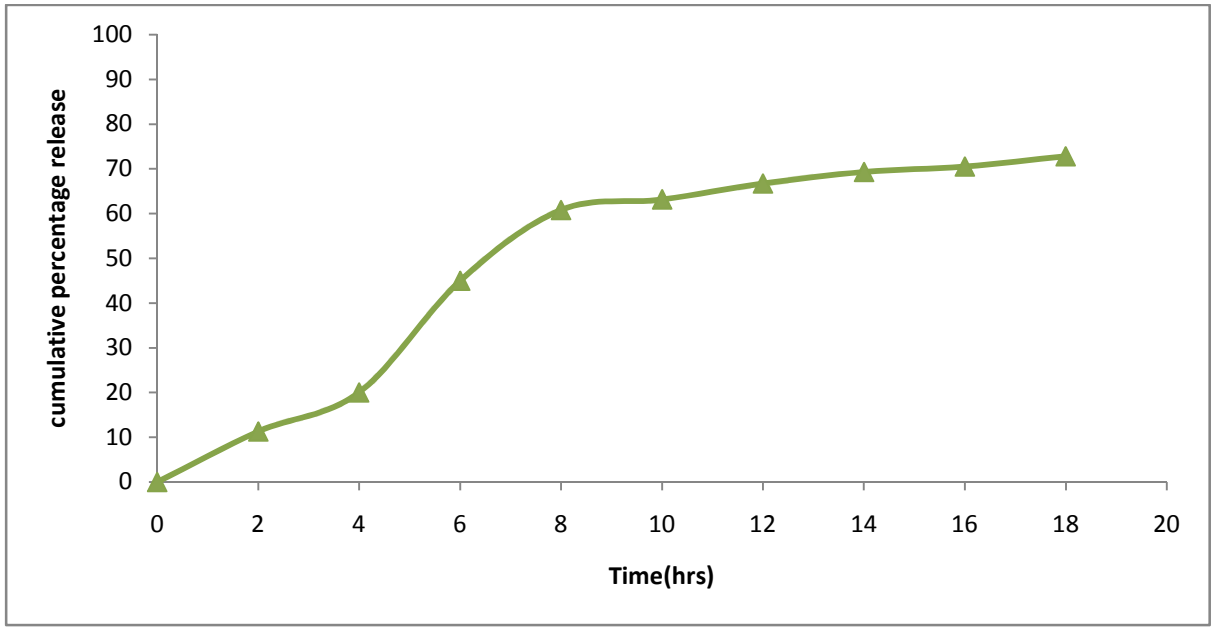


Table No.18

***In-vitro* Dissolution Profile For Formulation F4**

Time (Hrs)	Absorbance	Concentration	Amount in 900ml (mg)	Cumulative % Drug released
2	0.018	1.02	1.84	9.2
4	0.051	2.88	5.2	26
6	0.091	5.14	9.26	46.3
8	0.106	5.96	10.7	53.7
10	0.119	6.72	12.1	60.5
12	0.131	7.36	13.2	66.3
14	0.141	7.93	14.2	71.4
16	0.151	8.53	15.3	76.8
18	0.163	9.16	16.5	82.5

Graph No.9

Dissolution Profile of Formulation F4

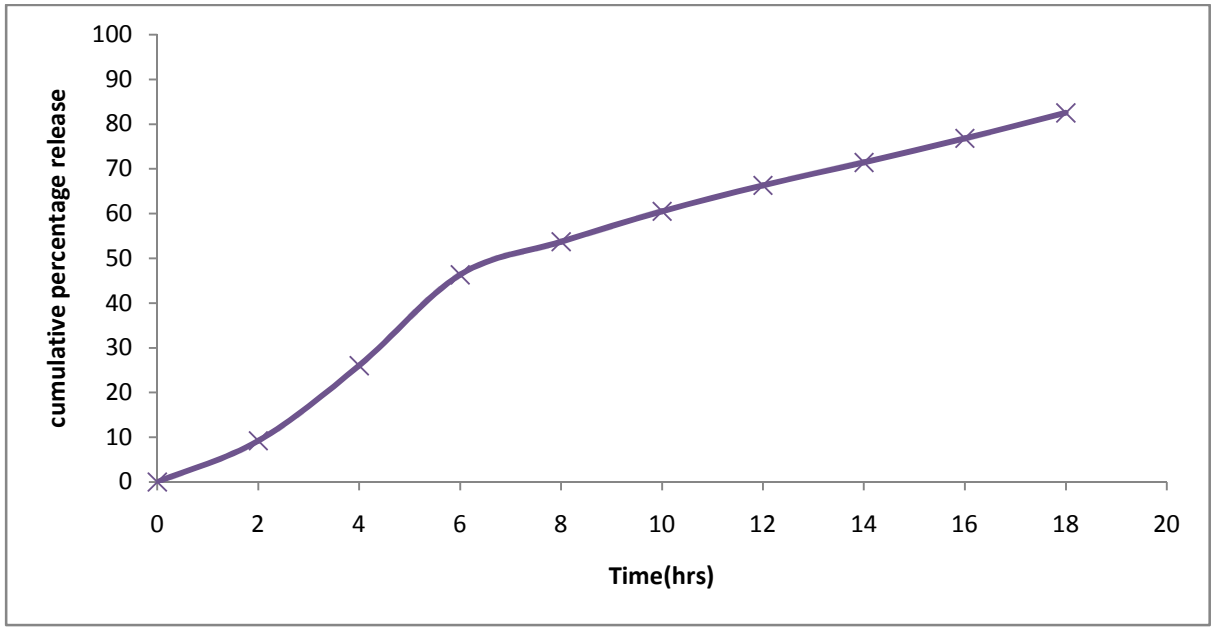


Table No.19

***In-vitro* Dissolution Profile For Formulation F5**

Time (Hrs)	Absorbance	Concentration	Amount in 900ml (mg)	Cumulative % Drug released
2	0.023	1.34	2.42	12.1
4	0.043	2.42	4.36	21.8
6	0.075	4.23	7.62	38.1
8	0.098	5.52	9.94	49.7
10	0.110	6.21	11.1	55.9
12	0.120	6.74	12.1	60.7
14	0.129	7.25	13.0	65.3
16	0.139	7.83	14.1	70.5
18	0.148	8.35	15.0	75.2

Graph No.10

Dissolution Profile of Formulation F5

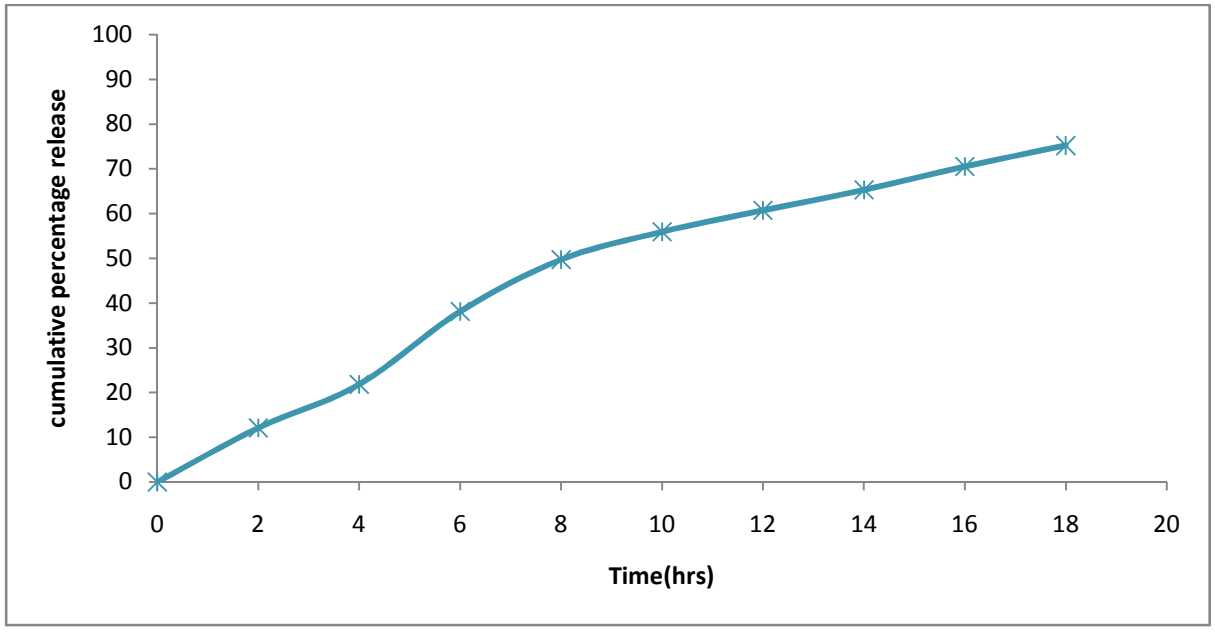


Table No.20

***In-vitro* Dissolution Profile For Formulation F6**

Time (Hrs)	Absorbance	Concentration	Amount in 900ml (mg)	Cumulative % Drug released
2	0.029	1.63	2.94	14.7
4	0.054	3.04	5.48	27.4
6	0.066	3.75	6.76	33.8
8	0.091	5.16	9.30	46.5
10	0.108	6.10	10.5	54.9
12	0.129	7.22	13	65
14	0.131	7.36	13.26	66.3
16	0.136	7.68	13.8	69.2
18	0.142	8.0	14.4	72.1

Graph No.11

Dissolution Profile of Formulation F6

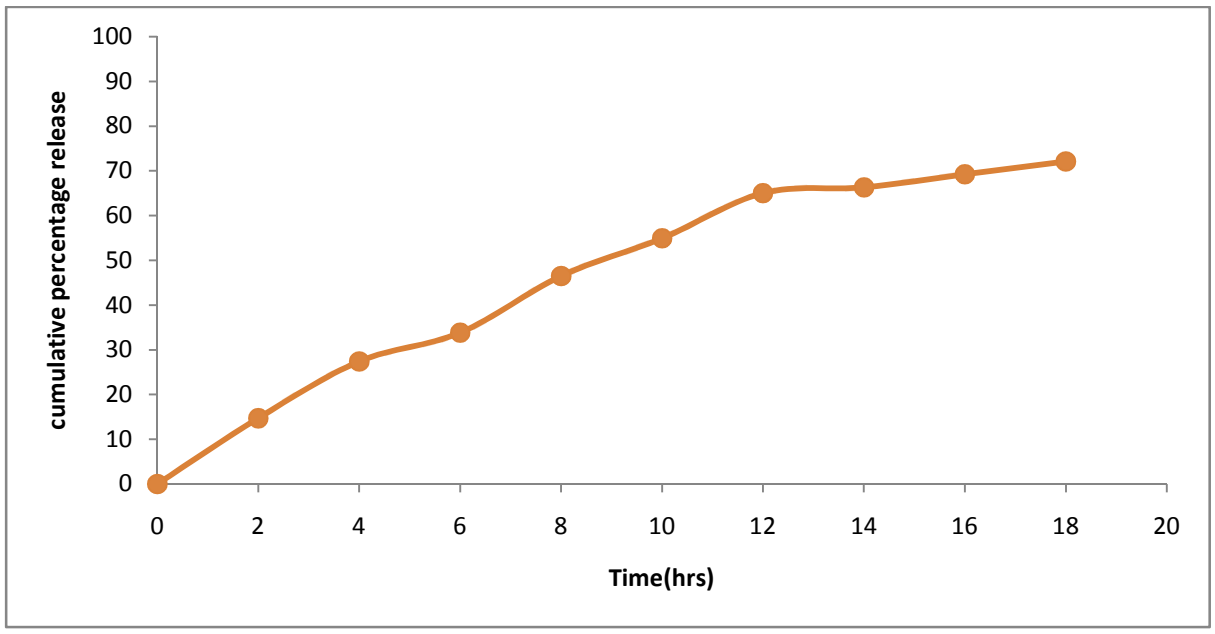


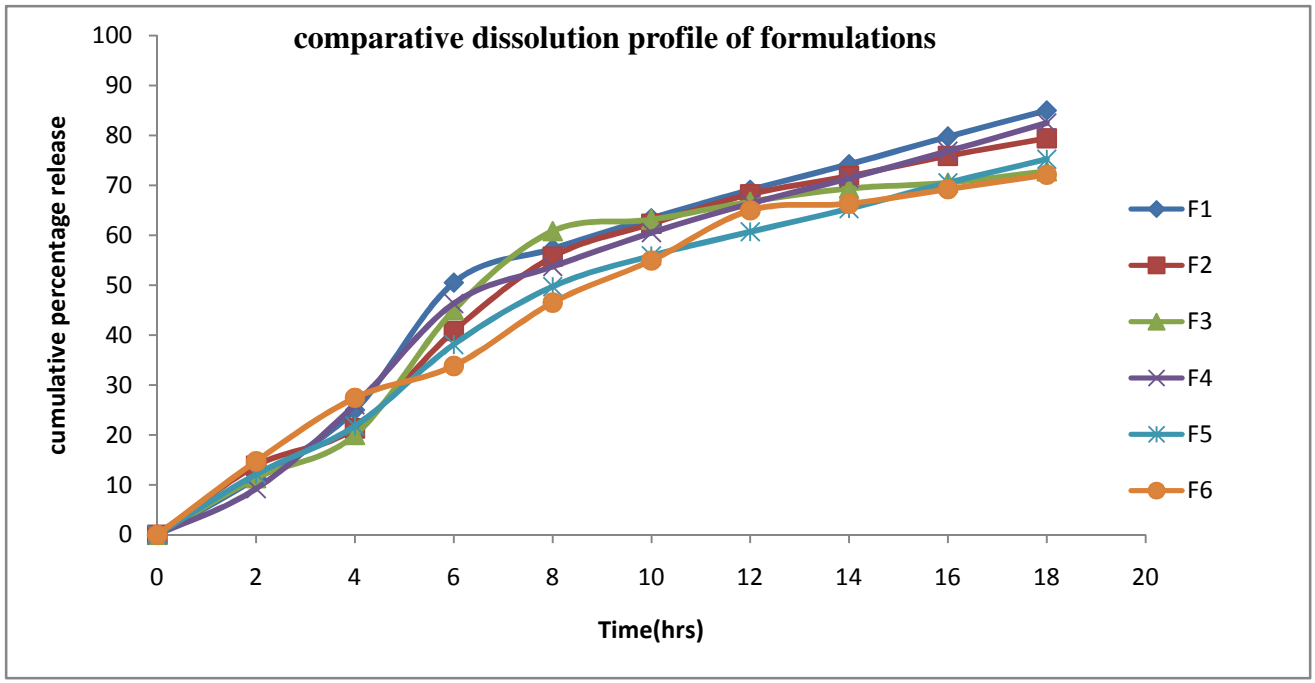
Table No.21

Comparative cumulative percentage release profile of formulations F1-F6

Time (hrs)	Cumulative percentage drug release (%)					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
2	11.3	13.8	11.3	9.2	12.1	14.7
4	25	21.3	20	26	21.8	27.4
6	50.5	40.8	45	46.3	38.1	33.8
8	57.3	55.7	60.8	53.7	49.7	46.5
10	63.4	62.3	63.1	60.5	55.9	54.9
12	69.1	68.2	66.7	66.3	60.7	65
14	74.2	71.8	69.3	71.4	65.3	66.3
16	79.7	75.9	70.5	76.8	70.5	69.2
18	85	79.4	72.8	82.5	75.2	72.1

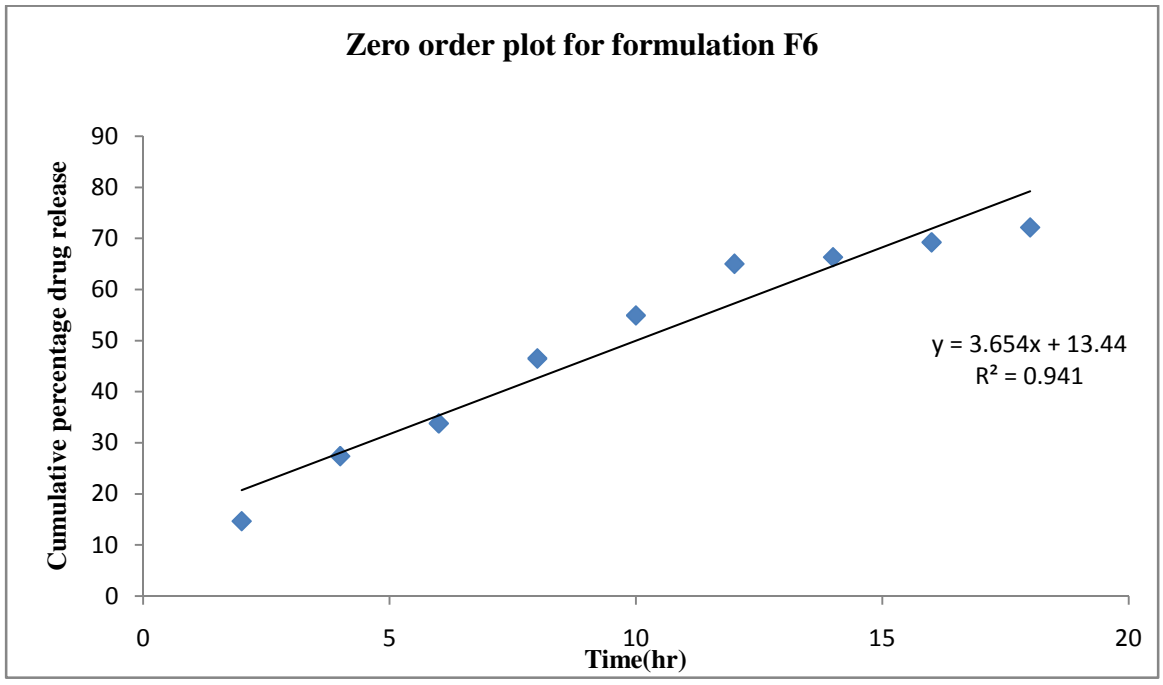
Graph no.12

Comparative dissolution profile of formulations

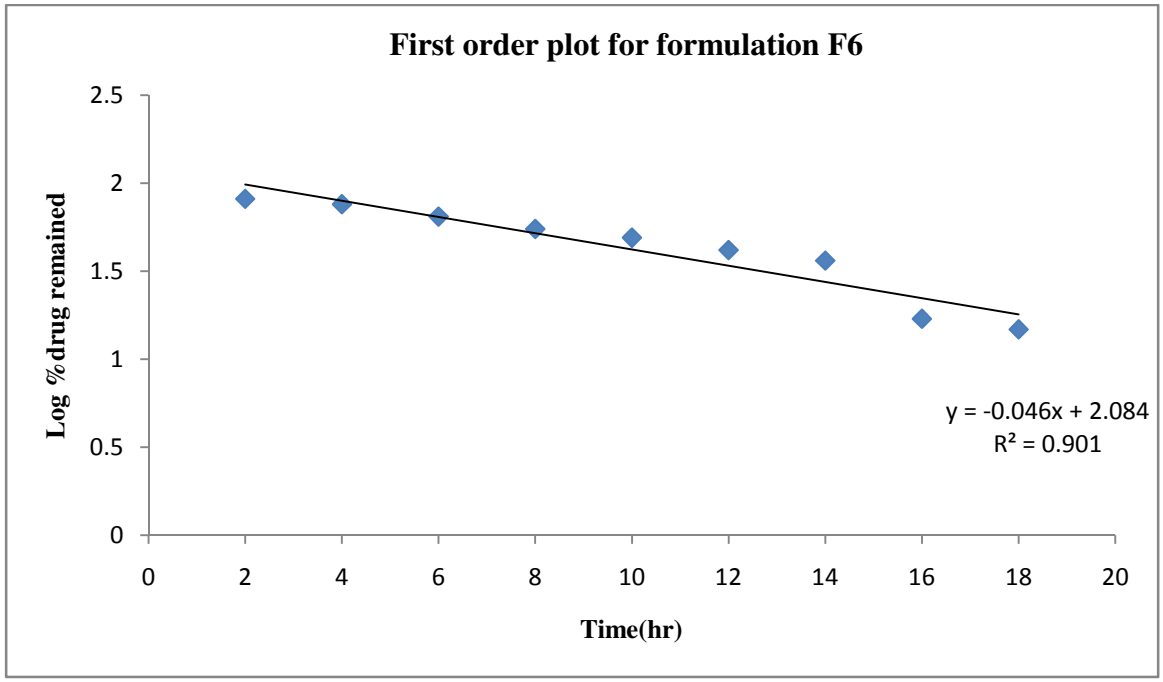


Cumulative Percentage Release of Drug Vs Time

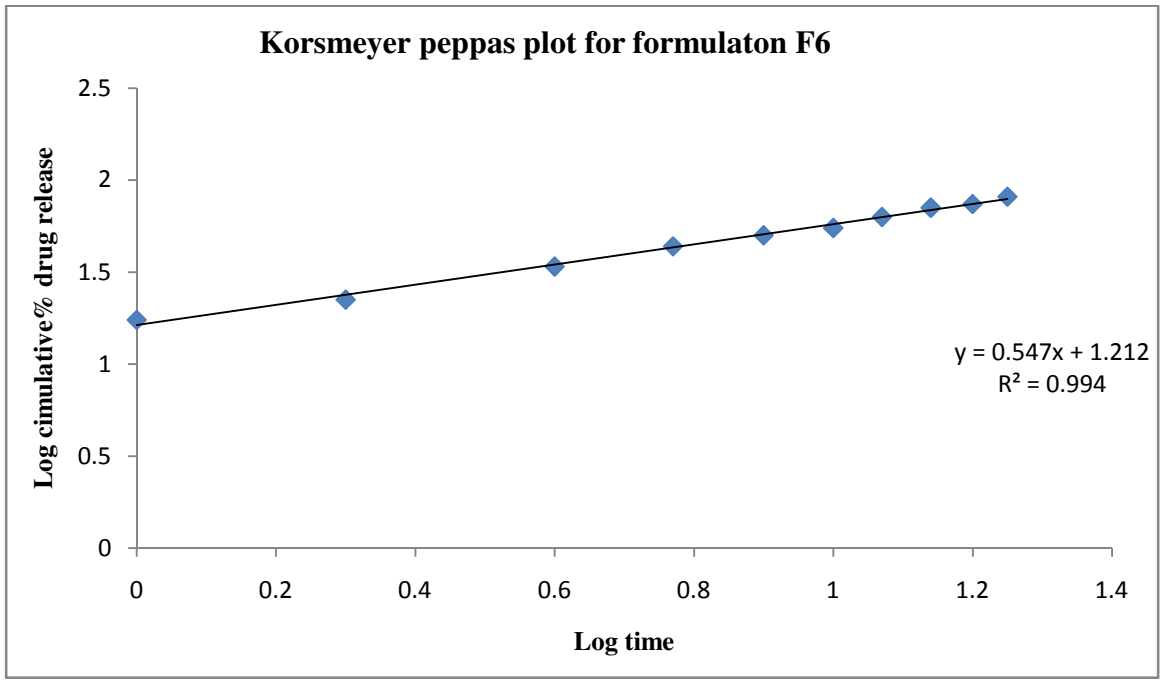
Graph No.13



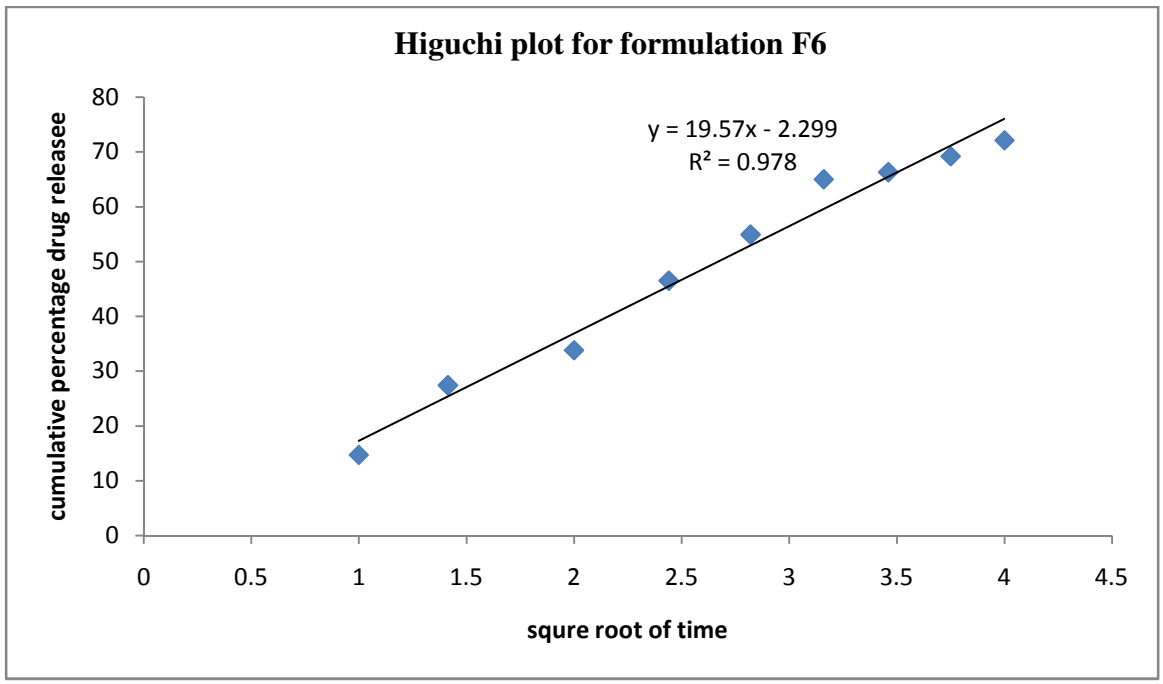
Graph No.14



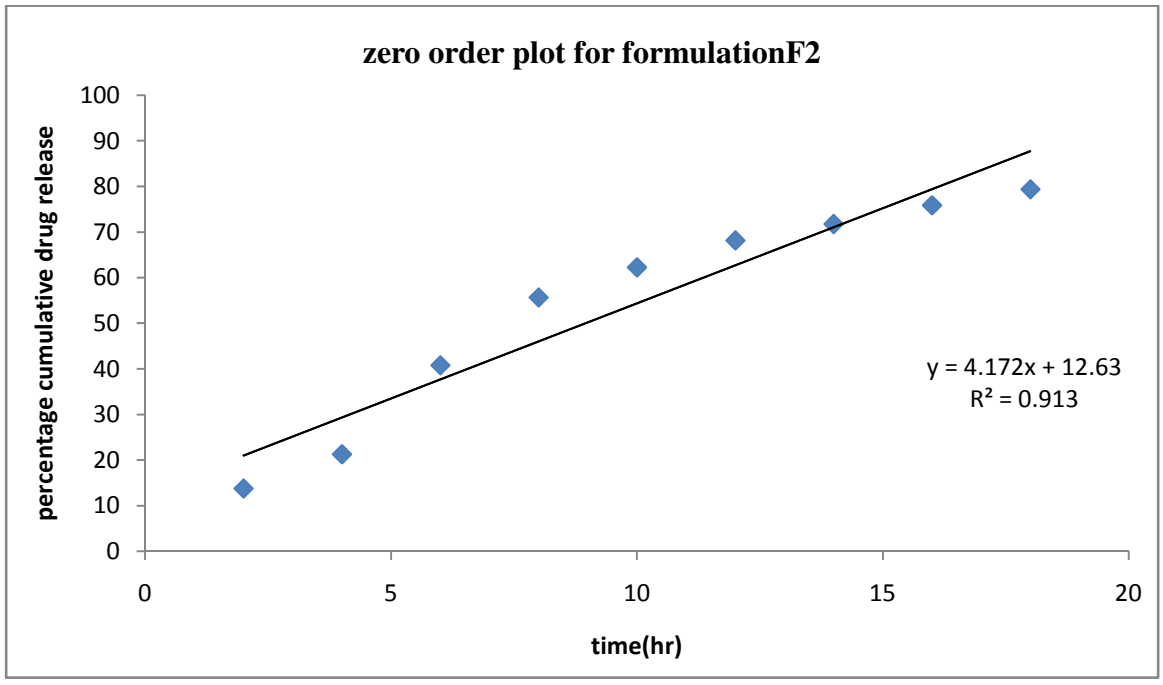
Graph No.15



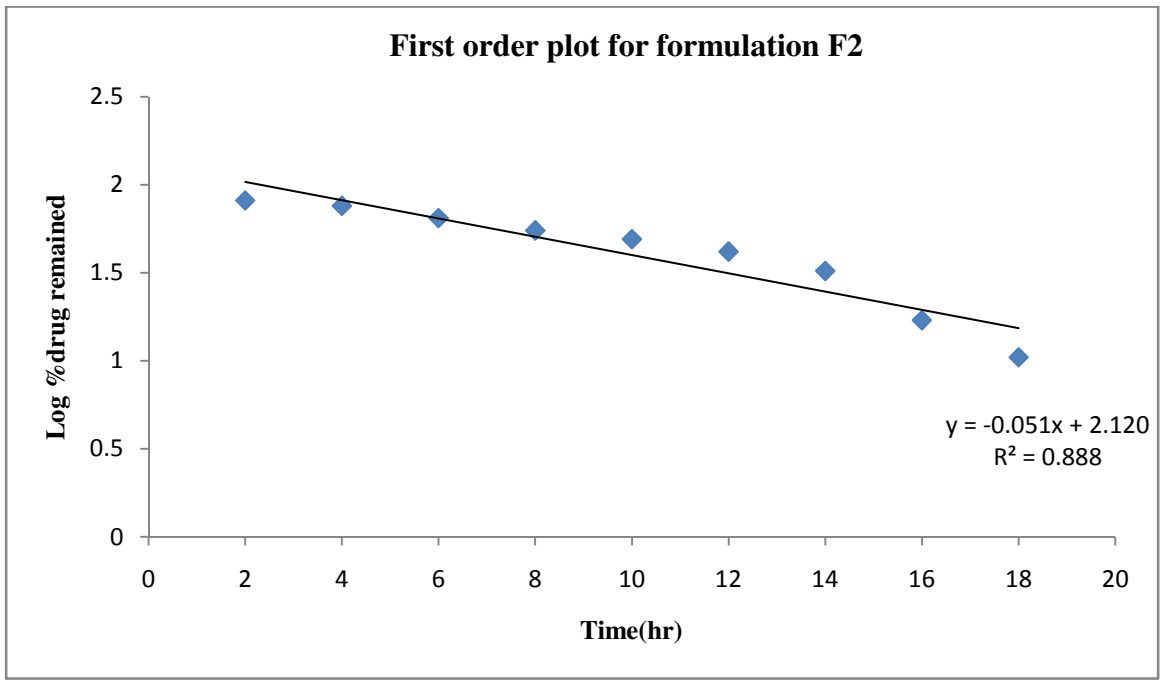
Graph No.16



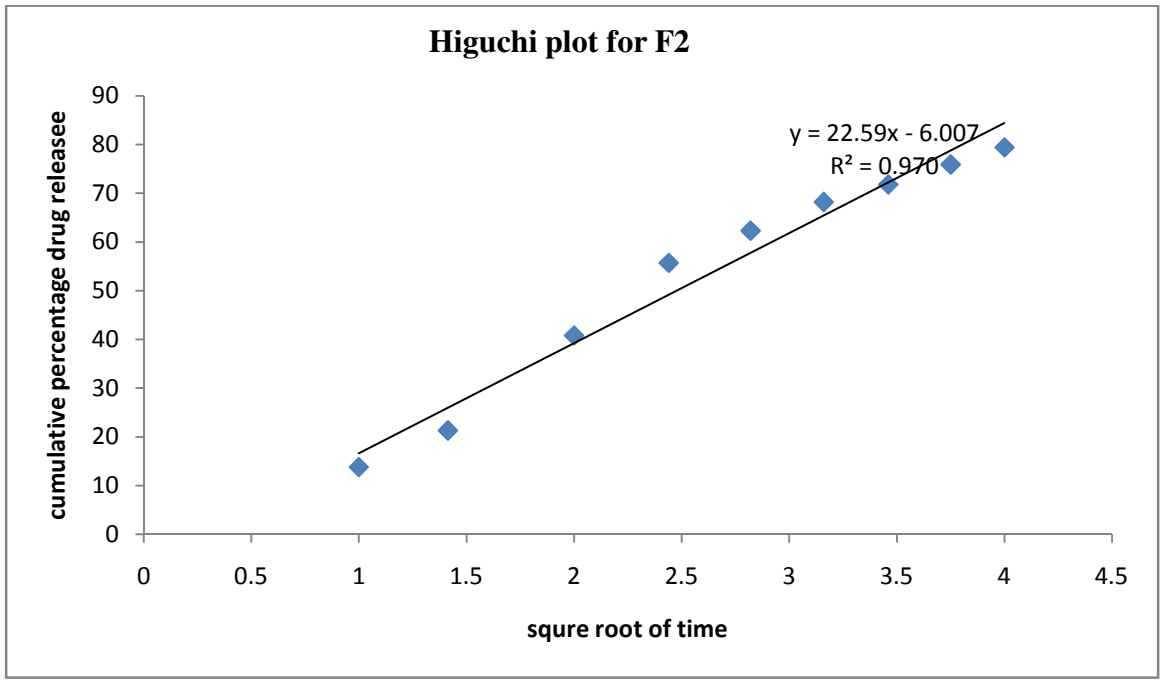
Graph No.17



Graph No.18



Graph No.19



Graph No.20

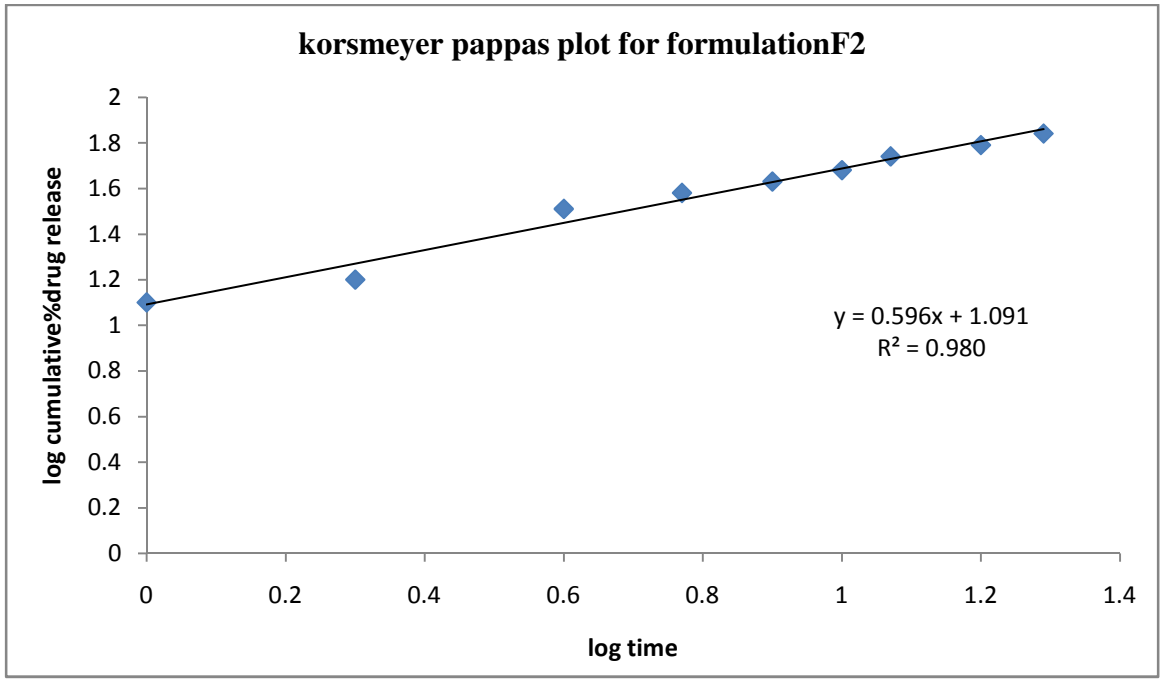


Table No.22

Kinetic Release Data of Formulations

Formulations	Zero order plots	First order plots	Higuchi plots	Korsmeyer-peppas plot		Possible drug release mechanism
	R^2	R^2	R^2	R^2	n	
F1	0.9618	0.9899	0.9908	0.9836	0.5892	Non-fickian, higuchi
F2	0.9136	0.8885	0.9701	0.9801	0.5962	Non-fickian, higuchi, first order.
F3	0.9604	0.9913	0.9882	0.9882	0.5847	Non-fickian, first order, higuchi.
F4	0.9414	0.9774	0.9768	0.9849	0.5834	Non-fickian, higuchi, first order.
F5	0.9666	0.8539	0.9950	0.9877	0.5728	Non-fickian, higuchi, first order.
F6	0.9416	0.9018	0.9782	0.9948	0.5475	Non-fickian, higuchi, zero order.

R^2= Regression coefficient n=slope

ACCELERATED STABILITY STUDIES

The formulations were stored in a oven at 37 ± 2˚C and 65% RH for a period of six weeks. The samples were analyzed for drug content every week by spectrophotometer at 258nm.

Method

Microspheres of formulations F1 and F5 were individually wrapped using aluminium foil and packed in ambered color screw cap bottle and put at above specified condition in incubator for 45 days. After 45 days microspheres were evaluated for percentage drug content.

Table No.23

Stability Studies of Percentage Drug Content of Formulation F1 & F5

Days	F1		F5	
	37℃&65%RH	60℃	37℃&65%RH	60℃
1	60.74	60.70	83.20	83.09
7	59.57	58.23	82.12	81.98
14	56.29	55.03	81.09	80.02
21	55.09	54.18	80.45	79.23
38	54.13	53.21	78.97	78.54
45	52.06	50.98	77.05	76.37

RESULTS AND DISCUSSION

In this present work efforts have been made to develop gelatin loaded microspheres of Lisinopril dihydrate using "Coacervation phase separation technique" using biogenic polymers gelatin, sodium alginate and carbopol.

In the present work, total six formulations were prepared and the detailed composition is shown in **Table No.6.** The prepared microparticles were subjected to granulometric study, angle of repose, scanning electron microscopy, drug entrapment efficiency, *In-vitro* dissolution and stability studies.

Micromeritic Properties of Microspheres

Flow properties of the prepared microspheres were determined by conducting Angle of repose, Bulk density determinations shown in **Table No.10, 11.** Angle of repose of F1, F4& F6 formulations are below 25 and shows excellent flow properties remaining are having good flow properties. The prepared Lisinopril Dihydrate microspheres were subjected to bulk density test and the result indicates good packing property. Among all formulations F6 has shown good micromeritic properties.

Drug Polymer Interaction (FTIR) Study

To check the compatibility of the drug with various polymers, IR spectra of the drug, polymers and combination of the drug and polymers were taken. The IR spectra of the drug and their combinations with polymers are shown in **Graph No. 2-5.** The characteristic major peaks of Lisinopril dihydrate were obtained at 3296.32 cm^{-1}, 3555cm^{-1}, 1658.99cm^{-1}, 1302.02cm^{-1} and 1420.69cm^{-1}. The values of peaks of drug and its combination with polymers were shown in the **Table No.14.** It was observed that all the major peaks of Lisinopril dihydrate were intact when it was in combination with polymers and no considerable changes in the IR peaks were observed. So FT-IR spectra indicate the stable nature of Lisinopril in combination with polymers.

Percentage Yield of Formulations

The percentage yield of microspheres of all the formulations was in the range of 78.90% to 88.53% shown in the **Table No.13.** The microspheres prepared by this method were found to be discrete, spherical, free flowing and it was observed by Scanning Electron Microscopy (SEM) **Figure No.3.**

Drug Entrapment Efficiency of Microspheres

The entrapment efficiency was in the range of 60.78 to 88.24% shown in **Table No12.** The formulations F6 and F5 were shown high entrapment efficiency of 83.24% & 82.76% which were made by combination of gelatin and sodium alginate polymers. The mean particle sizes of formulations were in the range of 67.9-76.4 µm. It was mentioned in **Table No.8**. The shape of the Microspheres shows smooth surface.

In vitro Drug Release Studies

Dissolution profile of gelatin containing Lisinopril dihydrate Microspheres in Phosphate buffer pH 7.4 showed that Micro spheres with low amount of gelatin released 85.0% (F1) of Lisinopril after 18 hrs while Micro spheres prepared with high amount of gelatin released only 79.4% (F2) of Lisinopril. The *In-vitro* Dissolution profile of gelatin-carbopol containing Lisinopril dihydrate Microspheres in Phosphate buffer pH 7.4 showed that Microspheres with high amount of carbopol were most effective in showing down the drug release 82.5% (F4), while the Micro spheres containing high amount of sodium alginate were released 72.1% (F6) of Lisinopril for 18 hrs at controlled rate. So F6 was taken as optimized formulation for its high entrapment efficiency and controlled release.

The data obtained in the in-vitro dissolution studies were grouped in to modes of data treatments as follows:

- Cumulative percent drug release v/s time (Zero order).
- Cumulative percent drug release v/s square root of time (Higuchi Matrix Model).
- Log cumulative percent drug retained v/s time (first order).
- Log cumulative percent drug release v/s log time (krosmeyer peppas model).

The results of the in vitro dissolution studies of formulations F1 to F6 are shown in **Table No.15-20**. The plots of cumulative percentage drug release v/s time, log cumulative percentage drug retained v/s time and cumulative percent drug release v/s square root of time, log cumulative percent drug release v/s log time were drawn and represented graphically. All the formulations exhibited anomalous (non-fickian) diffusion (n value is in between 0.5 to 1.0) mechanism. The drug release mechanism was diffusion controlled as the plot of higuchi model is linear.

Morphological Examination

Morphology of the microspheres was investigated by scanning electron microscopy. The photographs of formulations taken by scanning electron microscope were shown in the **Figures No.3 and 4**. The microspheres were spherical in shape and free flowing. The surface of microspheres was observed as smooth as shown in the **Figure.No.4.**

Stability Studies

Stability study was carried out for the formulations F1 and F5 at 37°C ± 1°C & 60° C for a period of 45 days and the results were noted in **Table No.23.** The decrease in the percentage yield of formulations F1 and F5 were less, after 45 days of stability studies and they were considered as stable products.

SUMMARY AND CONCLUSION

The study was undertaken to formulate and evaluate Lisinopril dihydrate microspheres using gelatin as a natural polymer and carbopol and sodium alginate in different proportions. Preformulation studies of Lisinopril were done initially and results directed for the further course of formulation. Based on the preformulation studies different batches were prepared using selected excipients. Prepared microspheres were evaluated for the % yield, drug content, Entrapment efficiency, Particle size determination, In-Vitro dissolution test and scanning electron microscopy, stability studies.

The drug content and entrapment efficiency were good. Among all the formulations F6 showed better results in controlled and slow release of drug from microspheres. Formulations showed controlled release for 24 hrs.

Dissolution was carried out in phosphate buffer pH 7.4 at 258nm. All the formulations were evaluated using different kinetic models i.e. Zero order kinetics, first order kinetics, korsmeyer peppas model and Higuchi kinetics. All the formulations exhibited Non fickian diffusion mechanism. The drug release was diffusion controlled as the plot of Higuichi model was found to be linear. F6 followed Zero order kinetics model.

Surface morphology and sphericity were also good. The formulation F6 was selected as an optimized formulation as it has high entrapment efficiency, good flow property, high percentage yield and following zero order kinetics with 72.1 % of drug release in 18 hours.

The Micro encapsulation of Lisinopril Dihydrate with gelatin, gelatin –carbopol and gelatin-Sodium alginate by co-acervation phase separation technique utilizing temperature change and cross linking with formaldehyde, was able to sustain the drug release efficacy. The microspheres having gelatine-sodium alginate and gelatin-carbopol polymers mixtures (F6&F3) provides the best sustained release formulations and other formulations (F1, F2) may be suitable for prolonged action formulations. These studies compared the release behaviour of gelatin, gelatin-sodium alginate and gelatin –carbopol controlled release system of Lisinopril microspheres. The F3 & F6 formulations had more pronounced controlled release effects compared with other formulations and F6 formulation is considered as optimised formulation among all formulations.

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